1967

Some effects of nickel on the microbial population of activated sludge

Virgil Eugene Carr

Department:

SOME EFFECTS OF NICKEL ON THE MICROBIAL POPULATION OF ACTIVATED SLUDGE

BY

VIRGIL EUGENE CARR -1935-

A

THESIS

submitted to the faculty of the

UNIVERSITY OF MISSOURI AT ROLLA

in partial fulfillment of the requirements for the

Degree of

MASTER OF SCIENCE IN CIVIL ENGINEERING

Rolla, Missouri

1967

Approved by

Sotirios G. Arhongelis (advisor) D. J. Wolff

Bobby A. Hyson Donald J. Sachs

ABSTRACT

The purpose of this investigation was to study the effects of nickel on the microbial population of activated sludge, to establish a relationship between effluent quality and changes in microbial population, and to determine the fate of nickel applied.

Five one liter fill and draw activated sludge units were fed settled domestic sewage and received constant daily nickel doses of 1-10 mg/l, and a 50 mg/l slug dose applied after 27-30 days of operation. The parameters used were the chemical oxygen demand (COD) of the effluent and membrane filtered effluent, mixed liquor and effluent total suspended solids, mixed liquor volatile suspended solids, mixed liquor and effluent nickel concentrations, mixed liquor and effluent microbial counts, and mixed liquor oxygen uptake.

It was found that nickel concentrations in the range of 1-10 mg/l: (a) caused an increase in effluent COD resulting from the presence of a large amount of organic suspended solids; (b) increased the number of bacteria, both dead and viable, discharged in the effluent; (c) discouraged the growth of rotifers (except for the 1 mg/l dose) and free swimming ciliated protozoa and encouraged the growth of stalked protozoa. About one-half of the nickel introduced was lost in the effluent, while considerably more was released following the addition of a slug dose; this could account for the recovery of activated sludge from slug doses. The absence of free swimming protozoa appeared to be responsible for the deterioration of the effluent.

ACKNOWLEDGMENT

The author wishes to express his appreciation to his advisor, Dr. S. G. Grigoropoulos, whose time and suggestions were given freely during the course of the study and preparation of this thesis, and to thank Professor J. Kent Roberts for his review of the thesis.

The research was supported in part by a Federal Water Pollution Control Administration Training Grant No. 1T1-WP-86-01A1. The author also wishes to give thanks to the Civil Engineering Department of the University of Missouri at Rolla for providing the necessary facilities which made this thesis possible.

The author's wife deserves much thanks for her understanding and patience shown during the long period required for the preparation of this thesis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (Continued)

I. INTRODUCTION

The detrimental effects of nickel on the activated sludge waste treatment process have been demonstrated by several investigators (1, 2, 3). The most noticeable effects appear to be reflected in increased effluent suspended solids and chemical oxygen demand. The toxicity threshold concentration* has been reported to vary from 1.0 to 2.5 mg/l of nickel.

Investigators at the Cincinnati Water Research Laboratory** of the Federal Water Pollution Control Administration (1, 2) have shown that the complete activated sludge treatment process removed only about 30 percent of the nickel in the influent sewage, while the remaining was lost in the plant effluent. Their investigations and those of Matthews (3) have also indicated that systems subjected to heavy slug doses of nickel were able to recover from the shock.

Few attempts have been made to find the cause of the increased effluent chemical oxygen demand and suspended solids in nickel fed activated sludge units. Matthews (3) has suggested that this increase might be related to the protozoan population of the sludge. He based this

---

*Daily concentration which can be added to activated sludge without significantly affecting effluent quality.

**Formerly the Robert A. Taft Sanitary Engineering Center.

suggestion on the works by McKinney and Gram (4) who have demonstrated that an activated sludge unit with protozoa gave a better quality effluent than one without, and by Hill (5) who reported some time ago that protozoa appeared to suffer from the presence of nickel in activated sludge.

Research is needed to establish whether there is a deficit of animal life in activated sludge subjected to nickel and to attempt to relate this shortage to the reported increased effluent suspended solids and chemical oxygen demand. The possibility of a correlation between the limited affinity of activated sludge microorganisms for nickel and their ability to recover from slug doses also needs to be studied.

The purpose of this investigation was to determine the effects of nickel on the microbial population of activated sludge; more specifically, to study the relationship between effluent quality, as measured by effluent suspended solids and chemical oxygen demand, and changes in microbial population. Also of interest was the determination of the fate of nickel by means of a complete metal balance.

A series of fill and draw activated sludge units, supplied with laboratory developed sludge, was used in this study. Constant daily nickel doses, and one slug dose toward the end of the run, were applied to the units following the daily feed of settled sewage. The parameters

used were the chemical oxygen demand of the effluent and the effluent filtered through a membrane filter; the effluent and mixed liquor total suspended solids; the mixed liquor volatile suspended solids; the effluent and mixed liquor nickel concentrations; the effluent and mixed liquor microbial counts; and the mixed liquor oxygen uptake measured by the Warburg respirometer.

II. LITERATURE REVIEW

The objective of this literature review was to study previous investigations pertaining to the effect of nickel on the microbial population of activated sludge. Although several articles have been written concerning the effects of nickel on the activated sludge treatment process, few have attempted to relate this to the microbial population or to changes in population. A complete review of the literature pertaining to the effects of nickel on activated sludge was presented by Matthews (3) in 1965. The present review is primarily concerned with the effects of nickel on the microbial population of the sludge, the ability of the sludge to recover from slug doses, and the fate of nickel in the activated sludge process.

An English investigator, Hill (5), who was in charge of a sewage works receiving plating wastes, reported in 1947 that his microscopic observations of activated sludge receiving nickel indicated that higher organisms in the sludge, especially stalked vorticella and free swimming paramecia, appeared to suffer from the presence of nickel.

In his studies, Hill employed two similar aeration vessels of approximately two liter capacity which were fitted with small air diffusers, seeded with activated sludge from a treatment plant, and fed with domestic sewage. Four soparate series of experiments were carried out, each

using a control and nickel concentrations of 1, 3, 6, and 10 mg/l, respectively, added as nickel sulfate in the sewage feed. The units were fed three times daily, with each study lasting four weeks. Following each feeding, the units were aerated for six hours and settled for two hours.

Hill did not report actual numbers of organisms, but presented his observations of the condition of the protozoa at the conclusion of each run; these observations are summarized in Table I. He also reported that the presence of nickel in activated sludge inhibited nitrification.

McDermott, Barth, and their co-workers (1, 2) at the Cincinnati Water Research Laboratory have extensively studied the effects of heavy metals on activated sludge. Nickel, in the form of nickel sulfate, was added in the sewage feed to continuous flow activated sludge units. Various constant doses, ranging from 1.0 to 10 mg/l, were applied and slug doses of 25, 50, and 200 mg/l were added for 4 hours to units which had been receiving a constant dose of 2.5 mg/l for a period of 14 days. The doses of 25 and 50 mg/l did not impose a very great stress on the system. However, the final effluent showed a marked increase in biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended matter, and turbidity 10 hours after the initiation of the 200 mg/l dose; these effects diminished in a rather linear manner during the following 30 hours, and the system was producing effluent of "preslug" quality 40 hours after

TABLE I
EFFECT OF NICKEL ON PROTOZOA PRESENT IN ACTIVATED SLUDGE*

EXPERIMENT NUMBER	NICKEL CONCENTRATION mg/l	OBSERVATIONS	
		CONTROL	NICKEL FED UNIT
1	1	Protozoa very active. Effluent very clear.	Protozoa fairly active. Effluent very clear.
2	3	Protozoa very active. Effluent very clear.	Protozoa active but certain forms rather sluggish (Vorticella and Carchesium). Effluent turbid at the first part of the run but cleared in about 10 days.
3	6	Protozoa very active. Vorticella extremely active. Carchesium also present in fair numbers. Effluent very clear.	Protozoa not very active, numerous dead organisms present. Effluent turbid. Took about 9 days to reach a clear effluent after stopping nickel dose in sewage feed.
4	10	Carchesium, Paramecium, Vorticella very active. Effluent very clear.	Very few protozoan types active and these mostly flagellates. Effluent had dense turbidity.

*After Hill (5).

the application of the slug dose. The addition of the 2.5 mg/l dose was continued after the 200 mg/l slug dose. The nickel content of the final effluent reached its peak 10 hours after the slug, and gradually dropped to its normal level after 60 hours.

The distribution of the nickel fed among the primary and excess activated sludge was determined for the 1.0 and 10 mg/l runs. It was found that primary treatment of sewage removed 3 to 5 percent of the incoming nickel and that the complete activated sludge treatment process removed only about 30 percent of the incoming nickel.

Barth, et al. (6) in a companion study which was primarily concerned with the effects of a combination of copper, chromium, zinc, and nickel on the efficiency of the activated sludge treatment process, observed that there was no nitrification in metal loaded systems. They believed that this lack of nitrification was due to the inability of nitrifying organisms to acclimate to the metals.

Stones (7), on the basis of results obtained from actual plant operation in England, reported that primary settling of sewage with a detention period of 12 hours removed about 20 percent of the incoming nickel. This is higher than the 3 to 5 percent value reported by McDermott, et al. (1) who, however, employed a detention time of only 1.2 hours. This would indicate that longer detention times

allow more nickel removal. Stones also reported that the activated sludge unit alone removed about 30 percent of the nickel, which is the same as the total nickel removal obtained by McDermott, et al. for the complete activated sludge treatment plant.

Moulton and Directo (8) used a continuous flow activated sludge unit fed with a synthetic sewage to study the effects of a slug dose of nickel. A 6 hour dose of 15 mg/l in the feed resulted in an increase of the effluent COD from 26 to 39 mg/l in one run, and from 32 to 42 mg/l in another run approximately 10 hours after the initiation of the nickel feed. The 10 hour time is the same as reported by McDermott et al. (1) for maximum effect; however, these investigators found that slug doses of 25 and 50 mg/l did not significantly affect a system acclimated to 2.5 mg/l of nickel. The units employed by Moulton and Directo (8) were not acclimated, but the maximum effect of the slug dose after 10 hours, as judged by the reported effluent COD, does not seem to be too great.

The Warburg respirometer has been employed by two groups of investigators in attempting to find the effects of nickel on activated sludge microorganisms which had not been previously acclimated to nickel. The results of these studies are summarized in Table II.

TABLE II
WARBURG RESPIROMETER STUDIES OF THE EFFECTS
OF NICKEL ON UNACCLIMATED ACTIVATED SLUDGE

INVESTIGATOR	SUBSTRATE	RUN LENGTH Hours	SLUDGE CONCENTRATION mg/l	NICKEL CONCENTRATION mg/l	REPRESSION OF UPTAKE* %
Dawson and Jenkins (9)	No substrate added, other than that present in the activated sludge.	2.5	1000	1 10 100	0 30 100
Heukelekian and Gellman (10)	Sewage added to give 70 percent by volume and sufficient sludge added to give a suspended solids concentration of 2000 mg/l.	22	2000	5 10 25 50 100	12 32 58 73 79

*As compared to a control to which nickel had not been added.

The studies by Dawson and Jenkins (9) demonstrated that the effect of nickel upon the oxygen uptake was produced almost at once. This was shown by using Warburg respirometer flasks with side arms, which enabled the addition of the metal 90 minutes after the beginning of the run, and observing that the uptake curves started to flatten out immediately after the addition of the nickel.

On the other hand, Heukelekian and Gellman (10) reported that whatever toxic effects were produced at the higher nickel concentrations they were not immediately apparent during the first hour following the metal addition. The oxygen utilization by samples containing 100 mg/l of nickel was practically equal to that of the control sample during the first hour, but as the run proceeded the differences became more noticeable. As shown in Table II, Heukelekian and Gellman used a 2000 mg/l sludge concentration and sewage as an additional substrate, while Dawson and Jenkins used 1000 mg/l of sludge and did not add an additional substrate. Heukelekian and Gellman believed that because of the higher solids concentration the time required for the metal to be absorbed into the floc was greater than if 1000 mg/l of solids had been used. They also found that the more diluted the sewage, the more pronounced were the toxic effects demonstrated by the nickel. These findings by Heukelekian and Gellman may explain the rapid action of nickel reported by Dawson and Jenkins.

All the investigations reported thus far in this review of the literature have dealt with either pilot plant studies or Warburg respirometer studies. It is also noted that the Warburg studies reported were for slug doses of nickel to unacclimated activated sludges. Matthews (3) was the first to report the use of both the Warburg respirometer and pilot plant studies in evaluating the effects of nickel on activated sludge. His primary objective was to determine the effect of nickel on activated sludge over a wide range of concentrations, and to evaluate the ability of the sludge to acclimate to the metal.

Matthews employed 6 one liter fill and draw activated sludge units which were fed daily with domestic sewage. One of the units did not receive any nickel and served as a control. To the remaining units steadily increased doses of nickel, in the form of nickel sulfate, were added and at the end of 21 days ranged from 4 to 64 mg/l in one run, while in another run the nickel concentration ranged from 2 to 8 mg/l after 18 days with a 30 mg/l slug dose administered on both the 19th and 20th day. For each run, effluent COD values were presented. Warburg respirometer studies were made with both acclimated and unacclimated sludge taken from the activated sludge units. The Warburg studies were performed using 10 ml of activated sludge, with the mixed liquor suspended solids adjusted to approximately 2430 mg/l, and 15 ml of fresh sewage containing an appro-

priate quantity of nickel. A wide range of nickel concentrations from 1.5 to 240 mg/l was studied.

Matthews observed a characteristic peak in the effluent COD curves determined in a number of batch units during his runs. The peak usually appeared sooner in units receiving higher nickel concentrations. After the peak, the effluent either improved or further deteriorated. He believed that this peak in effluent COD might have been caused by the death of less resistant species of microorganisms followed by the growth of species which could acclimate to nickel. These new microorganisms could have reduced the COD in the presence of a high nickel concentration, but when this concentration was exceeded, the microorganisms lost their ability to break down organic matter. Matthews reported that when some of the sludge units were subjected to a slug dose of 30 mg/l for two days, they were seriously inhibited but able to recover, and the activated sludges which had been exposed to lower nickel concentrations were less affected by the slug dose than the unexposed units.

Warburg respirometer runs revealed that for nickel concentrations ranging from 3.0 to 240 mg/l, the oxygen uptake was greater for units which had been exposed to small nickel doses than for units not previously exposed. This was particularly noticeable for nickel concentrations less than 60 mg/l.

Matthews also reported that large amounts of the nickel added were discharged with the effluent and that both acclimated and unacclimated sludges had little affinity for nickel.

He also noted the need for additional work in order to determine the effect of nickel on the microbial population of activated sludge, establish whether protozoa are present in nickel fed activated sludge, and define their role in the proper function of this treatment process.

III. MODE OF STUDY

The studies were performed using a series of fill and draw activated sludge units. Constant daily nickel doses followed with one slug dose were applied to the units immediately after the daily feed of strained settled sewage. Several parameters were used in evaluating the effects of nickel on the microbial population of activated sludge. The mode of study is presented in two parts: (a) activated sludge units and (b) parameters.

A. ACTIVATED SLUDGE UNITS

The sludge units consisted of 5 one liter graduated cylinders which were aerated with carbon filtered compressed air supplied through glass tubes extending nearly to their bottoms. The experimental arrangement is shown schematically in Figure 1. It was found necessary to cover the units with kraft paper to avoid algal growths caused by excessive exposure to sunlight. The units were maintained at room temperature and were fed at approximately the same time each day. Nickel was added to four of the units, while the fifth was maintained as a control with no nickel added. The daily procedure consisted of the following steps:

a. The solids stuck to the cylinder walls were scraped into the units with a rubber policeman.

b. The air was turned off and one of the aeration tubes was withdrawn and returned to allow any entrapped air to escape.

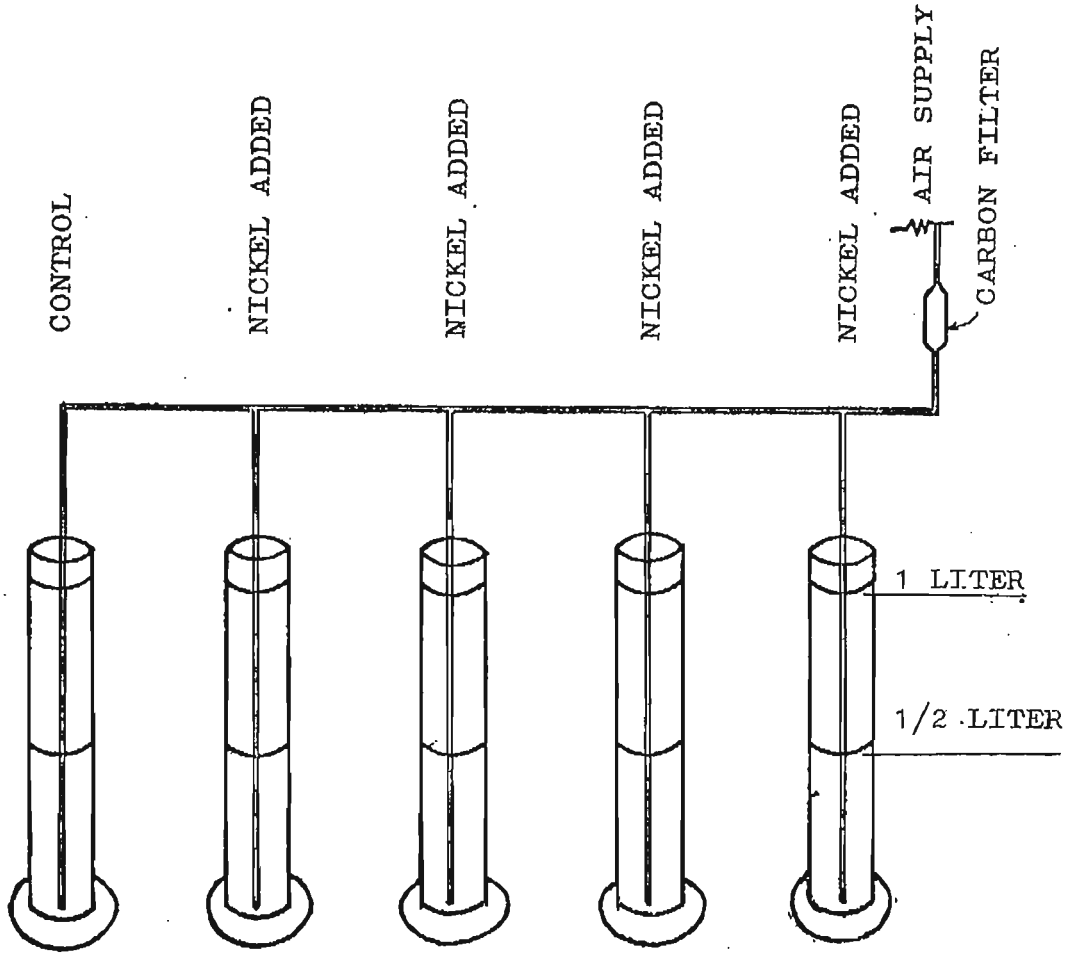


FIGURE 1. ACTIVATED SLUDGE UNITS

c. The sludges were allowed to settle for one hour.

d. The settled effluents were siphoned off to the 500 ml levels into 600 ml beakers from which appropriate samples were taken for chemical oxygen demand, suspended solids, and microbial counts. The remaining effluents were composited over a period of 3 or 4 days into separate one gallon bottles for nickel determinations and were stored in a walk-in incubator at 6°C.

e. Strained settled domestic sewage was added to each unit to raise its total volume to 1000 ml. The sewage was collected at the dosing tank of the Rolla Love Creek trickling filter plant. It was collected about once a week and refrigerated at 6°C.

f. The air was turned on.

g. Appropriate doses of a 2000 mg/l stock nickel solution were pipeted into the activated sludge units. The stock solution was prepared from reagent grade crystalline nickel sulfate ($NiSO_4 \cdot 6H_2O$).

h. The units were aerated for 23 hours.

B. PARAMETERS

1. Chemical Oxygen Demand.

The chemical oxygen demand (COD) was determined in the effluents of the sludge units and the effluents filtered through a membrane filter. The filtered effluent COD values were measured to establish how much of the effluent

COD was due to soluble organics and how much was due to suspended matter. The COD of the settled sewage, and of the sewage filtered through a membrane filter, was also determined periodically.

The procedure outlined in Standard Methods (11, p. 512) was used for this determination. It consisted of refluxing the sample with a mixture of silver sulfate, mercuric sulfate, potassium dichromate, and sulfuric acid for a period of 2 hours. The dichromate was used to oxidize the organic matter in the sample and convert it into carbon dioxide, water, and other compounds. The excess dichromate remaining after refluxing was measured with ferrous ammonium sulfate, and the amount used was employed in computing the COD value of the sample.

2. Total Suspended Solids.

Total suspended solids were determined in the mixed liquors and the effluents of the sludge units at frequent intervals. They were also measured periodically in the settled sewage used to feed the units.

This determination was made using the procedure described by Engelbrecht and McKinney (12) for activated sludge. Sample volumes of 20 ml for mixed liquors and 30 ml for the effluents and settled sewage were vacuum filtered through a tared membrane filter with openings

of 0.45±0.02 μ. The filter was dried, weighed, and the increase in weight was used to compute the total suspended solids.

3. Volatile Suspended Solids.

Volatile suspended solids determinations were made occasionally on the mixed liquors of the activated sludge units using a modification of the procedure outlined in Standard Methods (11, p. 425). The sludge was scraped off from the membrane filters which had been used for total mixed liquor suspended solids determinations into crucibles which had been dried at 103°C for one hour, cooled in a desiccator for 30 minutes, and tare weighed. The combined weight of crucible and sludge was determined to find the amount of sludge in the samples, and the crucibles and sludge were fired at 600°C for 15 minutes. They were then cooled in a 103°C drying oven for at least one hour and in a desiccator for 30 minutes. The weight of the crucible and remaining sludge was determined and used to compute the amount of solids lost during combustion.

4. Nickel Concentration.

Nickel concentrations were measured to determine the amount of nickel which was lost in the effluents and the amount of nickel which was present in the mixed liquors. All effluents from the units were saved, except for the amounts used for the determination of COD, suspended solids,

and microbial counts, and composited for 3 or 4 days. Nickel was determined in the composite effluent sample, and in grab samples of the mixed liquor collected while it was under aeration. By knowing how much nickel had been added to the systems, how much had been removed in the effluents, and how much was present in the mixed liquors, it was possible to arrive at nickel balances which were made once or twice a week.

With some modifications, nickel determinations were made using the alpha-furildioxime method outlined by Taylor (13) and modified by McDermott, et al. (1) and Matthews (3). The procedure used consisted essentially of the following steps. Ten ml samples of the effluents and mixed liquors were subjected to nitric and sulfuric acid digestion as prescribed in Standard Methods (11, p. 468) in order to destroy the organic matter present. In the digestion process, Hengar granules were used in place of glass beads or carborundum chips to catalyze the oxidation of the organic matter while also minimizing bumping. The digested samples were diluted to 100 ml; an appropriate volume was taken from each sample and its pH was adjusted to about 2 with 25 percent sodium hydroxide with the aid of a pH meter. The volumes used were usually 10 ml for the effluents, and 10 ml or less for the mixed liquors; in some cases it was necessary to reduce the mixed liquor volumes to as low as 2 ml because of the large amount of nickel

accumulated in the sludge. In each case when less than 10 ml of sample were used, enough deionized water was added to make 10 ml total. After pH adjustment the samples were transferred to 250 ml separatory funnels; 0.05 ml of 1 N potassium dichromate and 5 ml of 10 percent sodium citrate solutions were added to complex any iron present, and were followed with 0.1 gram of sodium thiosulfate added to reduce the possible interference of copper. Then, 0.06 ml of a 50 percent aqueous-alcohol solution of alpha-furildioxime and 0.5 ml of concentrated ammonium hydroxide were added to form a yellow-colored complex with nickel. The color complexes containing nickel were extracted with three 7 ml portions of chloroform. The separatory funnels were shaken 200 times following each addition of chloroform. The solvent extracts were removed after each shaking and combined in a 50 ml graduated cylinder. After the final extraction, the contents of the graduated cylinder were diluted to 25 ml with chloroform, mixed, and the percent transmittance was measured as soon as possible with a Bausch and Lomb Spectronic 20 spectrophotometer at 435 mμ wave length. A reagent blank was prepared using the same procedure as for the samples except that 10 ml of distilled water was used in place of the 10 ml sample volume. The percent transmittance was converted to optical density which was used with a previously prepared calibration curve to compute the quantity of nickel in the sample.

There are variations in the literature concerning the pH at which the digested nickel containing samples should be before adding the reagents for the chloroform extractions. Taylor (13) recommended that the sample should be slightly acid (not less than a pH of 1), McDermott, et al. (1) reported that the pH should be adjusted to 8-9, while Matthews (3) adjusted it to between 2 and 5 with sodium hydroxide in the presence of a methyl orange indicator. The latter method was used in the beginning of this study and it was found that in some samples color was not developed by the alpha-furildioxime and ammonium hydroxide. Upon checking, the pH of the solutions was found to be higher than was expected. It is believed that with the high strength of the 25 percent sodium hydroxide solution, and because the end point for methyl orange is pH 4.5, the critical point was passed in several instances. Using the pH meter and titrating with 25 percent sodium hydroxide to pH 2 worked out very satisfactorily. It was also found that at pH 1 no color was developed by the complexing chemicals.

This study indicated that there was a straight line relationship between nickel and optical density in the nickel concentration range of 0-15 µg, but the straight line proportionality from 15-20 µg was questionable.

5. Protozoan and Rotifer Count.

Protozoa and rotifers in the mixed liquor were counted using a Sedgwick-Rafter counting cell. This cell had a carefully calibrated area of 10 square centimeters and held a volume of exactly one ml. The 100x power of a clinical Bausch and Lomb microscope (Model LK4303) was used and a Whipple disk was placed in the eyepiece to facilitate counting. The Whipple disk was calibrated with a stage micrometer. The numbers of different types of protozoa and of rotifers were determined in several microscopic fields. Knowledge of the area of the square covered by the Whipple disk and of the total area of the counting cell enabled the determination of the numbers of the microscopic animals present in each ml of sample.

6. Bacterial Count.

This determination gave an indication of the total numbers of bacteria in the effluents, both dead and alive. The numbers of bacteria in the effluents were determined using stained slides of a carefully measured volume of sample spread over a known area. The slides were prepared by placing one drop of the effluent from a calibrated eye dropper on a slide which had been cleaned with Bon Ami and flamed to remove surface oil. The drop was spread over an area of about one square centimeter and allowed to air dry. Upon drying the smear was "fixed" by passing the slide rapidly through the flame of a Bunsen burner, film side

up, two or three times. The smears were flooded with several drops of methyl violet dye (one percent solution) which was allowed to remain 30-45 seconds before washing in tap water, using a gentle, indirect stream, to remove the excess stain. The slides were then air dried. The oil immersion lens with 970x magnification was used for viewing the slides. A Whipple disk was employed and was calibrated with a stage micrometer. By knowing the volume of samples and the area which they occupied on the slides and the area covered by one microscopic field, it was possible to calculate the numbers of bacteria per ml by counting the numbers of bacteria present in several microscopic fields. Undoubtedly certain extraneous particles were also counted on the stained slides as bacteria.

7. Standard Plate Count.

Standard plate counts were performed on the effluents from the units to determine the numbers of live bacteria present. They were performed according to Standard Methods (11, p. 592). An incubation temperature of 35°C was used for Run 2 and a 20°C temperature was used for Run 3. One ml of an appropriate dilution of effluent sample was placed in a Petri dish. Fifteen to twenty ml of melted sterile tryptone glucose extract agar taken from a 45°C water bath was poured on top of each sample and mixed. After the agar had hardened, the plates were incubated

inverted at the appropriate temperature. The bacterial cells were trapped in the agar and formed colonies which were visible to the naked eye after one or two days and could be counted with the aid of a Quebec counter.

8. Oxygen Uptake.

The oxygen uptakes of the mixed liquors of the various units were determined using a Gilson Medical Electronics Warburg respirometer (Model RWBP3) at different time intervals to provide a measure of the condition of the activated sludge units. Oxygen uptakes were measured on samples taken prior to the feeding of the units, and without an additional source of substrate added.

The test procedure included the following steps:

a. One ml of 10 percent potassium hydroxide was pipeted into the center well of each flask.
b. Twenty five ml of activated sludge from the appropriate unit were added to duplicate flasks.
c. Twenty five ml of settled sewage were added to each of two flasks to evaluate the strength of the sewage fed to the units, and 25 ml of distilled water were added to two other flasks which served as thermo-barometers and enabled compensation for atmospheric pressure variations.
d. The Warburg flasks were connected to the manometers and placed in the 20°C water bath with the stopcocks

open to the atmosphere. Heated vaseline seals were used for watertight connections.

e. The flasks were allowed to reach equilibrium by shaking at 80 to 82 oscillations per minute in the 20°C water bath for 5 minutes with the stopcocks open to the atmosphere.

f. The levels of the manometers were adjusted to the 150 mm mark and the stopcocks were closed, severing the connection of the flasks with the atmosphere.

g. Readings were taken at appropriate time intervals.

h. The flasks were reaerated whenever it was necessary.

i. At the end of the run the flasks were removed, their contents were discarded, and they were washed with tap water, dried, washed with chloroform, rinsed with tap water, dried, cleaned with potassium dichromate cleaning solution, rinsed with tap water and distilled water, and dried. They were then ready to be used again. The ground glass connections between the flasks and the manometers were cleaned with chloroform.

j. The manometer readings were used to compute the oxygen uptakes of the flasks at different time intervals using the following formula (14, p. 398):

$$\text{mg/l Oxygen Uptake} = 1{,}430 \frac{k}{v} h$$

Where: v = volume of sample (in ml)

h = change in pressure in manometer corrected for atmospheric pressure differences (in cm)

k = flask constant

The gas volumes of the closed systems were found by the water method described by McKinney (15, p. 109).

IV. PRESENTATION OF RESULTS

The effects of nickel on the microbial population of activated sludge were investigated in three separate experimental runs to enable the use of a wide range of nickel concentrations and to evaluate the reproducibility of data. Constant daily doses ranging from 1 to 10 mg of nickel per liter of mixed liquor were applied among the three runs, and a slug dose of 50 mg/l was applied in Runs 2 and 3.

At the beginning of each run, the five units were filled with laboratory developed activated sludge. Four of these units received constant daily nickel doses which were reached over a period of several days. The fifth unit, which was used as a control, received no nickel and was operated in the same manner as the other four. Any deviations that the nickel fed units had from the control were considered to be caused by the presence of the metal. Prior to the addition of the slug dose, one-half of the control was transferred to a sixth unit and this enabled one-half of the control to be slug dosed, while the other half still served as the control.

A. EXPERIMENTAL RUN 1

Run 1 served as a means of evaluating the procedures and determinations employed in the study, and for selecting

appropriate nickel doses for following investigations. The four nickel fed units received constant daily doses of 2, 3, 4, and 5 mg/l of nickel, respectively. The lower value was selected because it was believed by previous investigators (1, 2, 3) to be near the toxicity threshold level, and the higher values were thought to be capable of exhibiting noticeable toxic effects. Data were collected for a period of 46 days, and a slug dose was not applied. The parameters employed in this run were primarily the effluent COD values, the protozoa and rotifer counts, and the effluent and mixed liquor suspended solids.

The nickel feed schedule for this experimental run is shown in Table III. The constant nickel doses were reached in increments in order to allow for acclimation of the microorganisms to nickel.

The effluent COD values are shown in Table IV and Figure 2. All the nickel fed units were affected by the presence of nickel and, in general, the effluent COD increased with nickel concentrations.

The mixed liquor suspended solids in all units increased throughout the run (Table V and Figure 3). This was attributed to the high strength of the sewage fed to the units, as indicated by its COD value which was usually around 350 mg/l. At the end of the 46 day period, the suspended solids in the control unit were considerably higher than in the test units.

TABLE III
NICKEL FEED SCHEDULE
EXPERIMENTAL RUN 1

TIME Days	CONTROL UNIT	2 mg/l UNIT	3 mg/l UNIT	4 mg/l UNIT	5 mg/l UNIT
	NICKEL ADDED, mg/l				
0	0	0.5	0.5	0.5	0.5
1	↓	1.0	1.0	1.0	1.0
2	↓	1.5	1.5	1.5	1.5
3	↓	2.0	2.0	2.0	2.0
4	↓	↓	2.5	2.5	2.5
5	↓	↓	3.0	3.0	3.0
6	↓	↓	↓	3.5	3.5
7	↓	↓	↓	4.0	4.0
8	↓	↓	↓	↓	4.5
9	↓	↓	↓	↓	5.0
↓	↓	↓	↓	↓	↓
46	0	2.0	3.0	4.0	5.0

TABLE IV
EFFLUENT CHEMICAL OXYGEN DEMAND
EXPERIMENTAL RUN 1

TIME	COD, mg/l				
Days	CONTROL UNIT	2 mg/l UNIT	3 mg/l UNIT	4 mg/l UNIT	5 mg/l UNIT
4	30	30	30	30	30
11	9	38	28	28	38
19	75	103	94	178	168
23	92	71	122	133	112
26	37	92	111	102	139
30	37	83	--	--	211
32	--	--	--	--	90
35	27	36	45	72	108
37	45	55	109	91	127
46	44	44	88	96	88

TABLE V
TOTAL MIXED LIQUOR SUSPENDED SOLIDS
EXPERIMENTAL RUN 1

TIME	SUSPENDED SOLIDS, mg/l				
Days	CONTROL UNIT	2 mg/l UNIT	3 mg/l UNIT	4 mg/l UNIT	5 mg/l UNIT
12	1730	1640	1550	1710	1790
23	2070	1940	1800	1910	2140
30	2270	2130	2120	2170	2260
34	2450	2100	2070	2420	2340
37	2500	2060	2180	2360	2300
41	2530	2140	2130	2210	2150
46	2890	2540	2250	2520	2380

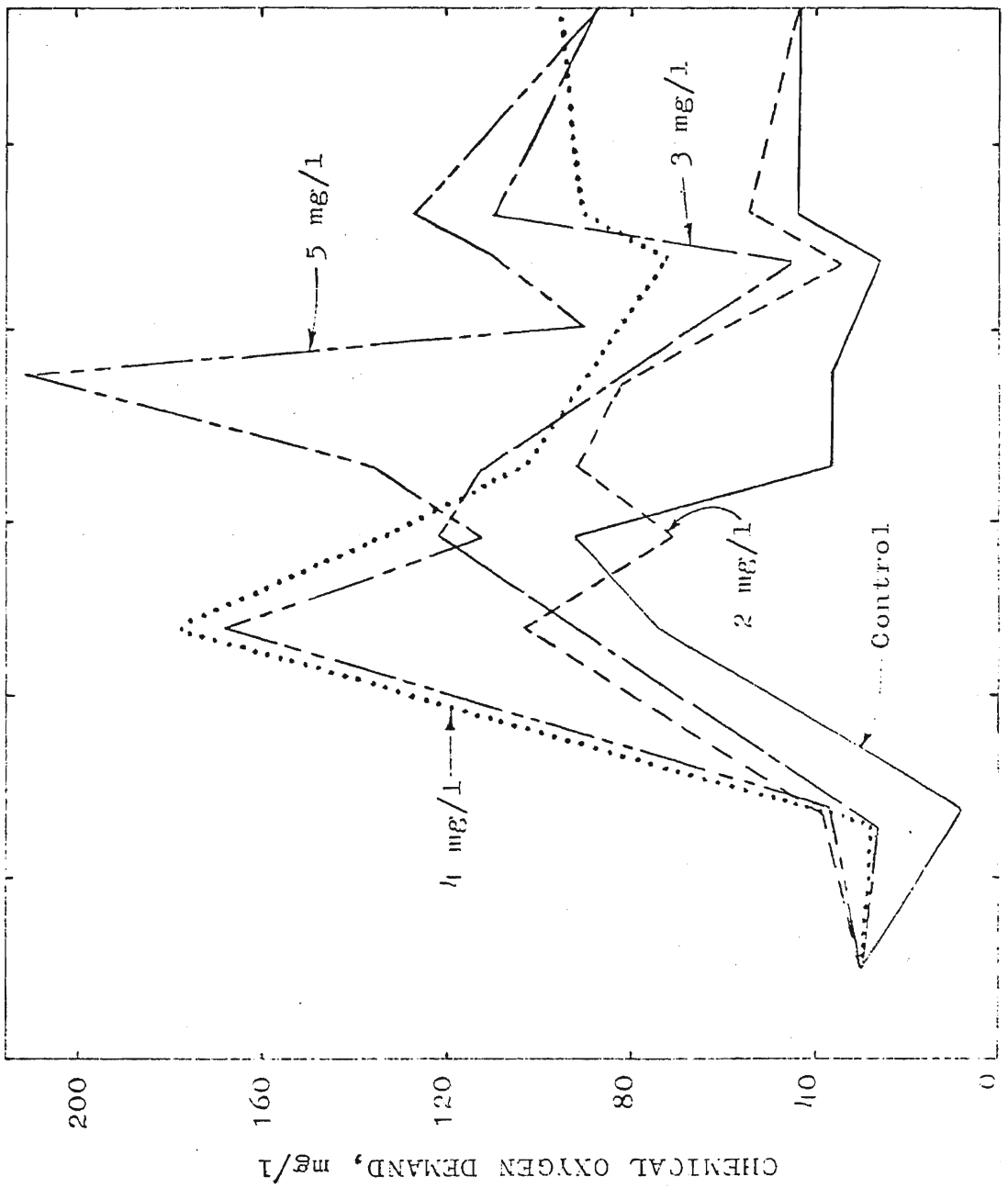


FIGURE 2. EFFLUENT CHEMICAL OXYGEN DEMAND
EXPERIMENTAL RUN 1

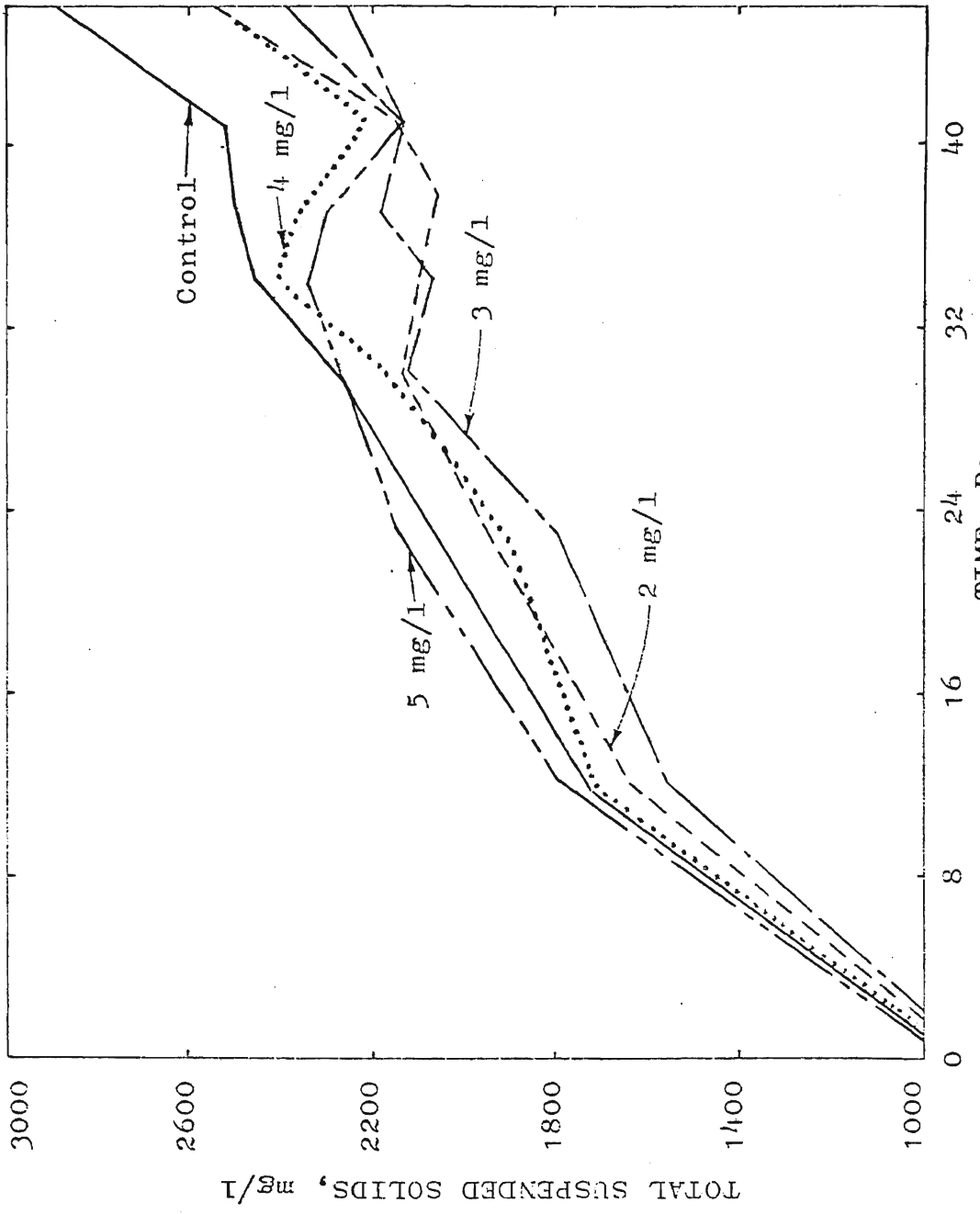


FIGURE 3. TOTAL MIXED LIQUOR SUSPENDED SOLIDS
EXPERIMENTAL RUN 1

The 4 and 5 mg/l units developed a visibly turbid effluent after 19 days of operation. The time of this observation corresponded to the peak in the effluent COD values of the same units. Effluent suspended solids determinations made on the 37th day of operation gave values of 40, 40, 70, 110, and 160 mg/l for the control, 2 mg/l, 3 mg/l, 4 mg/l, and 5 mg/l units, respectively.

The numbers of stalked protozoa and rotifers found in the mixed liquors are presented in Table VI and plotted in Figure 4. These two forms were the only animals continually observed in measureable numbers in this run. The stalked protozoa observed were Vorticella and colonial forms*. All types of rotifers were counted as one group. As can be seen in Figure 4, from the 25th day on to the end of the run the protozoan population in the control was very low, while the population in all the nickel fed units remained about the same with a few peaks. With the exception of the observations on the 10th and 22nd day, the protozoan population in the control remained lower than that in the nickel fed units. On the contrary, the rotifer population (Figure 4) was substantially higher in the control than in the nickel fed units. A few rotifers were observed throughout the run in the 2 and 3 mg/l units, but rotifers were practically nonexistent in the 4 and 5 mg/l units after 22 days.

*Several cells attached to a common stalk.

TABLE VI
PROTOZOAN AND ROTIFER COUNT
EXPERIMENTAL RUN 1

TIME	COUNT, Number/ml									
	CONTROL UNIT		2 mg/l UNIT		3 mg/l UNIT		4 mg/l UNIT		5 mg/l UNIT	
Days	ROTI-FERS	STALKED PROTOZOA	ROTI-FERS	STALKED PROTOZOA	ROTI-FERS	STALKED PROTOZOA	ROTI-FERS	STALKED PROTOZOA	ROTI-FERS	STALKED PROTOZOA
10	250	680	136	250	91	91	159	182	136	136
17	380	137	46	76	167	167	23	227	--	--
22	855	450	58	305	15	131	0	174	0	276
25	450	87	29	189	15	232	0	188	15	304
27	610	29	0	217	44	290	0	290	0	420
32	435	15	58	160	15	246	0	246	0	217
34	812	87	15	217	29	276	0	566	0	275
36	566	0	43	145	0	551	0	275	0	188
41	507	0	102	160	15	406	0	450	0	203
46	595	0	0	44	0	203	0	203	0	116

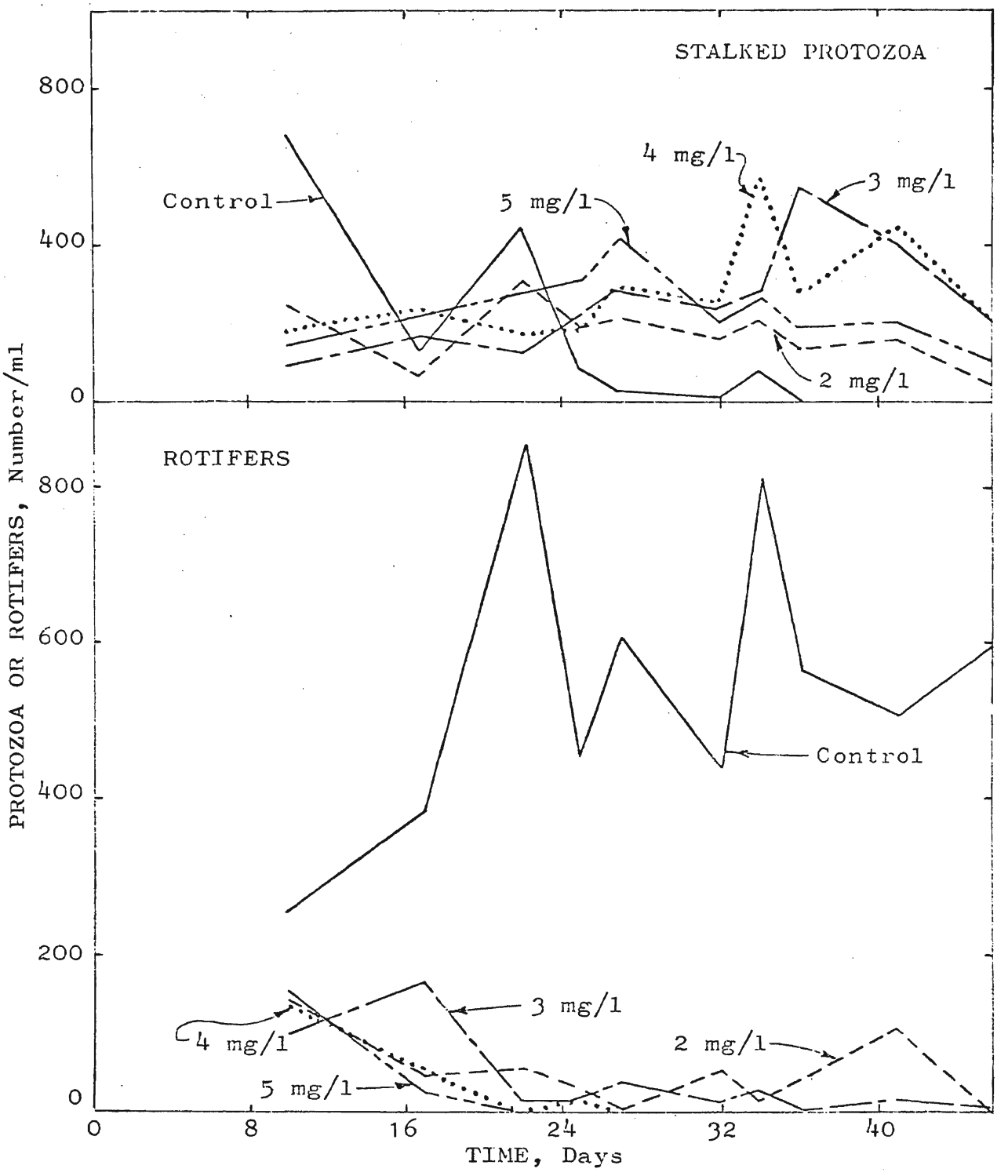


FIGURE 4. PROTOZOAN AND ROTIFER COUNT
EXPERIMENTAL RUN 1

B. EXPERIMENTAL RUN 2

Nickel was applied according to the nickel feed schedule shown in Table VII. Progressively increasing amounts of nickel were added until final constant daily doses of 2, 4, 7, and 7 mg/l had been reached. On the 30th day of operation all units, including one-half of the control, were subjected to a 50 mg/l slug dose. The nickel concentration range of 2 - 7 mg/l was selected in an attempt to duplicate the results of the 2 and 4 mg/l units employed in the previous run; and to evaluate the effects of a nickel dose which was higher than the 5 mg/l maximum dose used in Run 1. Duplicate 7 mg/l concentrations were used to evaluate the reproducibility of the study.

The effluent and filtered effluent COD data are shown in Table VIII and plotted in Figure 5. The effluent COD values of the control unit were well below the corresponding values of the nickel fed units. Also, the filtered effluent COD's were considerably lower than the unfiltered values, and the data for the nickel fed units were grouped closely together. The maximum filtered effluent COD in the nickel fed units was 60 mg/l and in the control 40 mg/l. The slug dose applied on the 30th day did not appear to have greatly affected the filtered effluent COD's. The effluent COD's indicated that nickel deteriorated the quality of the effluent substantially. Both the effluent and filtered effluent COD's of the duplicate 7 mg/l units remained very

TABLE VII
NICKEL FEED SCHEDULE
EXPERIMENTAL RUN 2

TIME	CONTROL*		2 mg/l	4 mg/l	7 mg/l	7 mg/l
	NO SLUG	SLUG	UNIT	UNIT	UNIT	UNIT
Days	NICKEL ADDED, mg/l					
0	0		0.5	0.5	0.5	0.5
1			1.0	1.0	1.0	1.0
2			2.0	2.0	2.0	2.0
3				4.0	4.0	4.0
4					7.0	7.0
28			2.0	4.0	7.0	7.0
29	0		0	0	0	0
30	0	50	50	50	50	50
31		0	0	0	0	0
40	0	0	0	0	0	0

*One-half of the control received a 50 mg/l slug dose on the 30th day.

TABLE VIII
EFFLUENT AND FILTERED EFFLUENT CHEMICAL OXYGEN DEMAND
EXPERIMENTAL RUN 2

TIME	COD, mg/l											
	CONTROL* NO SLUG		CONTROL* SLUG		2 mg/l UNIT		4 mg/l UNIT		7 mg/l UNIT		7 mg/l UNIT	
Days	EFF.	FILT. EFF.	EFF.	FILT. EFF.	EFF.	FILT. EFF.	EFF.	FILT. EFF.	EFF.	FILT. EFF.	EFF.	FILT. EFF.
2	101	--			55	--	51	--	59	--	30	--
4	--	31			--	34	--	34	--	48	--	44
8	44	--			117	40	150	51	146	40	172	55
15	47	26			156	43	102	38	121	43	106	43
19	50	--			101	--	67	--	101	--	113	--
22	48	40			95	48	91	60	143	56	147	56
30	60	35			108	43	95	56	138	39	134	61
31	35	27	75	27	120	44	93	27	111	22	120	27
34	31	9	88	26	88	18	70	40	106	18	114	22
37	29	25	136	41	95	41	70	33	157	50	132	41

*One-half of the control received a 50 mg/l slug dose on the 30th day.

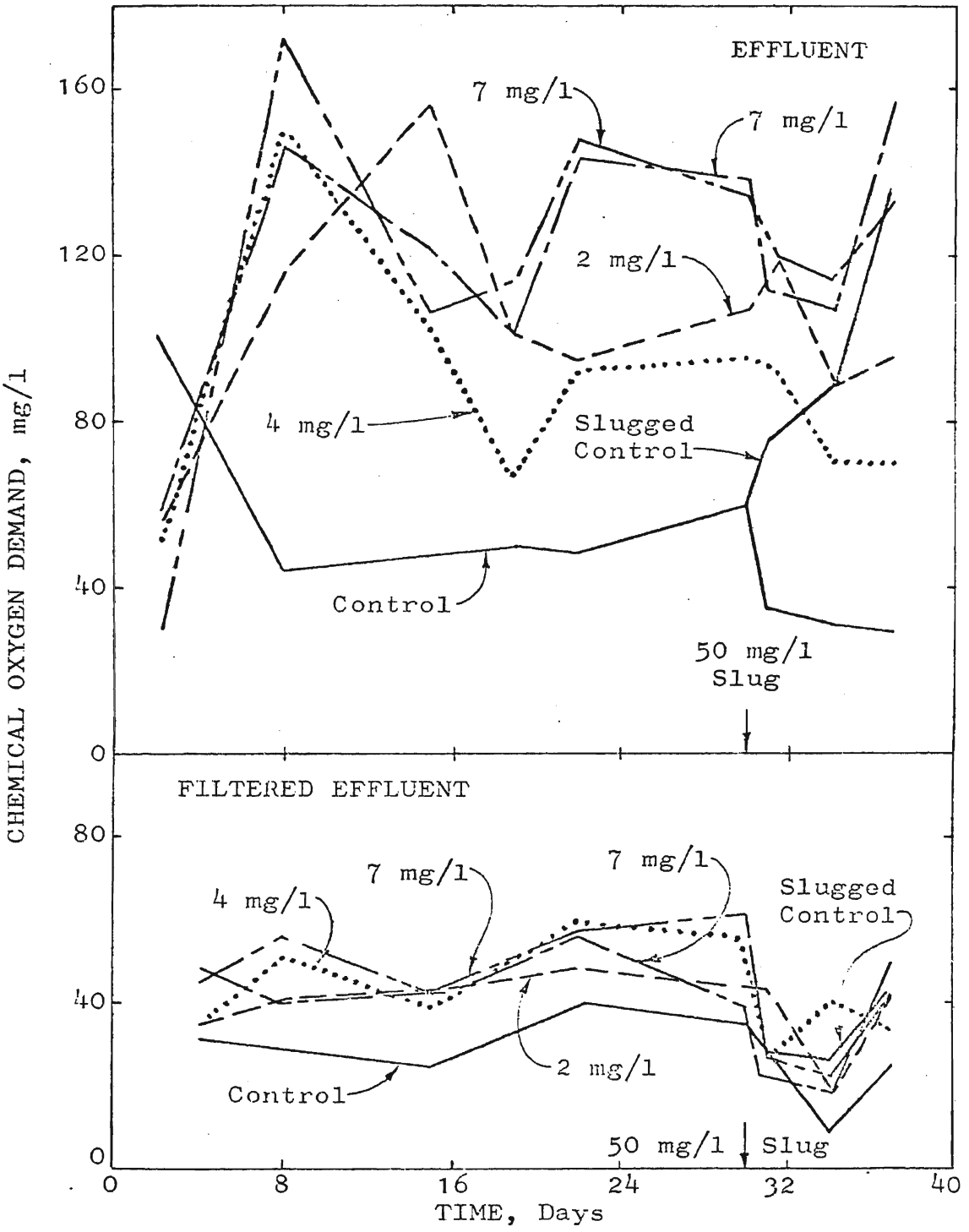


FIGURE 5. EFFLUENT AND FILTERED EFFLUENT CHEMICAL OXYGEN DEMAND EXPERIMENTAL RUN 2

close throughout the run. The addition of the slug dose affected the control much more than the nickel fed units. Seven days after the slug dose had been applied, the slugged control effluent COD was greater than those of the two units fed the lower nickel doses and almost as high as the two 7 mg/l units. This would support the finding by Matthews (3) that a system can become acclimated with small doses of nickel and be able to withstand slug doses.

The data for the effluent and mixed liquor suspended solids are presented in Table IX and in Figure 6. A comparison of the effluent suspended solids curves in Figure 6 with the effluent COD curves in Figure 5 indicated a definite similarity in the relative positions of the curves for the various units. The curve for the control was considerably below the curves for the other units. As with effluent COD's, the slug load did not affect the effluent suspended solids of the nickel fed units to any extent; however, it was very detrimental to the control unit. Six days after the application of the slug dose the effluent suspended solids of the slugged control were higher than the 2 and 4 mg/l units.

The total mixed liquor suspended solids (Figure 6) decreased almost constantly through the run. This was attributed to the decreasing strength of the settled sewage which was used to feed the units (Table X), and its inability

TABLE IX
TOTAL EFFLUENT AND MIXED LIQUOR SUSPENDED SOLIDS
EXPERIMENTAL RUN 2

TIME	SUSPENDED SOLIDS, mg/l											
	CONTROL* NO SLUG		CONTROL* SLUG		2 mg/l UNIT		4 mg/l UNIT		7 mg/l UNIT		7 mg/l UNIT	
Days	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR
0	--	2440			--	2480	--	2520	--	2510	--	2440
4	--	2540			--	2660	--	2570	--	2740	--	2550
5	40	--			66	--	80	--	80	--	88	--
8	40	2290			115	2400	135	2270	120	2320	165	2340
11	--	2460			120	2270	140	2180	90	2220	110	2320
12	56	--			116	--	148	--	160	--	140	--
15	33	2130			130	2070	77	2230	90	2260	77	2300
18	60	2440			93	2130	67	2370	53	2420	80	2400
22	30	2530			57	2080	93	2180	100	2490	110	2360
25	27	2630			73	1810	73	2190	117	2370	120	2350
30	57	2485			103	1830	77	1925	120	2150	123	2095
31	30	2185	60	2270	113	1695	73	1895	117	2105	107	2005
34	20	1705	70	1865	77	1510	93	1505	103	1835	107	1695
36	8	1340	88	1630	64	1290	44	1350	124	1635	92	1620

*One-half of the control received a 50 mg/l slug dose on the 30th day.

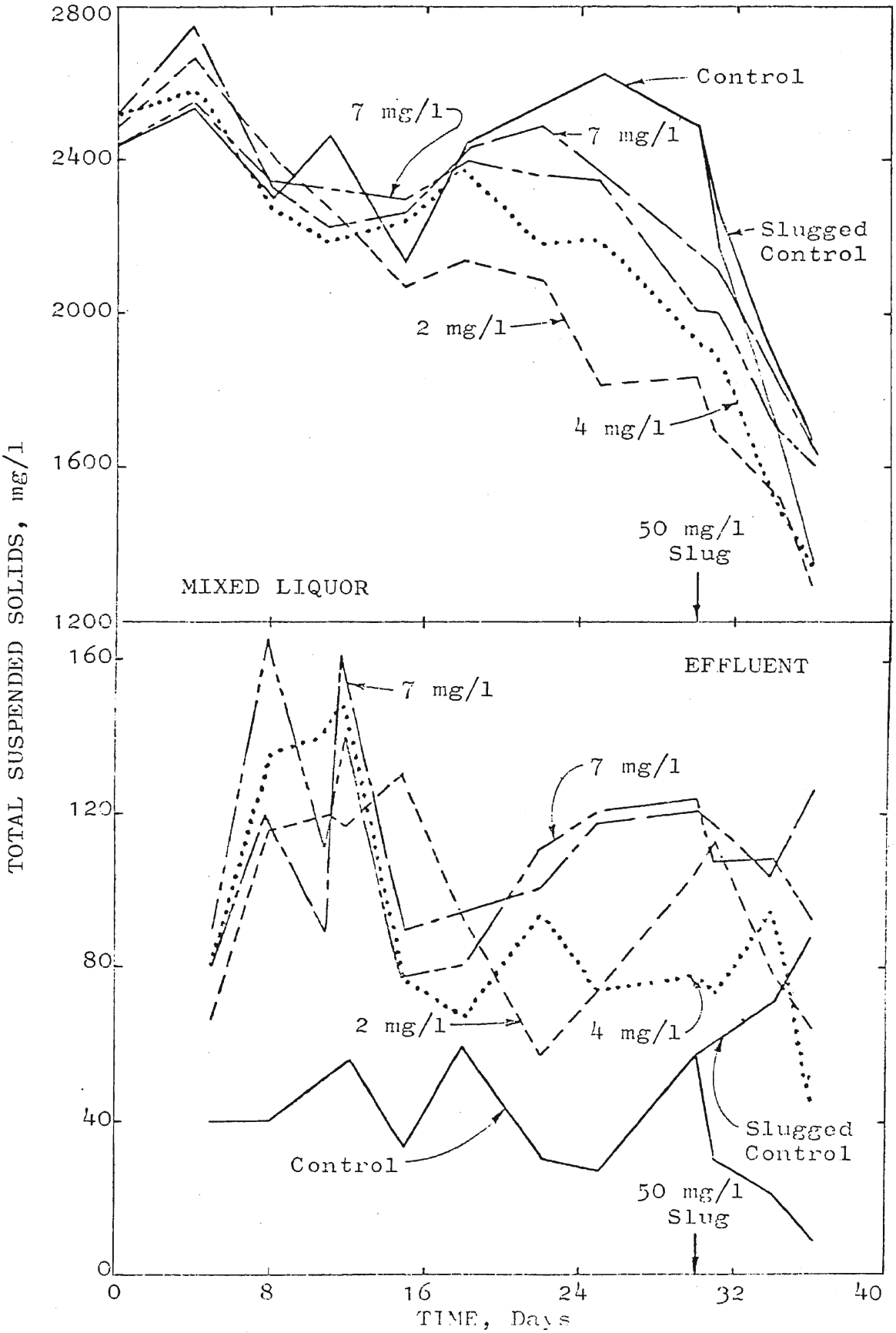


FIGURE 6. TOTAL EFFLUENT AND MIXED LIQUOR SUSPENDED SOLIDS EXPERIMENTAL RUN 2

TABLE X
SETTLED SEWAGE CHARACTERISTICS
EXPERIMENTAL RUN 2

TIME PERIOD Days		SEWAGE CHARACTERISTICS, mg/l		
		CHEMICAL OXYGEN DEMAND		TOTAL SUSPENDED SOLIDS
FROM	TO	SEWAGE	MEMBRANE FILTERED SEWAGE	
0	1	480	--	--
2	8	230	--	--
9	14	309	--	20
15	20	203	106	68
21	27	180	47	48
28	33	86	39	18
34	40	212	75	68

TABLE XI
VOLATILE SUSPENDED SOLIDS
EXPERIMENTAL RUN 2

TIME	VOLATILE SOLIDS, %					
	CONTROL*		2 mg/l	4 mg/l	7 mg/l	7 mg/l
Days	NO SLUG	SLUG	UNIT	UNIT	UNIT	UNIT
4	77		75	70	74	72
25	66		67	64	65	64
36	69	71	64	65	65	60

*One-half of the control received a 50 mg/l slug dose on the 30th day.

to sustain the high concentration of solids present at the beginning of the run. The strength of the sewage decreased for the first 33 days of the run, with the lowest COD value being 86 mg/l. The volatile mixed liquor suspended solids also decreased during the study period (Table XI) indicating a lower percentage of organic matter in the sludge and, consequently, an increased degree of endogenous respiration.

The data presented thus far indicate that the largest part of the increased COD in nickel fed units was caused by suspended matter and not by soluble organics. The nickel fed units continued to remove large fractions of the COD in the feed, but the suspended solids in the effluent were generally higher than those in the raw waste.

The numbers of bacteria in the effluents, as determined by microscopic count, are shown in Table XII and Figure 7. For the most part, more bacterial cells were present in the nickel fed units than in the control. Standard plate counts presented in Table XIII and Figure 8, verified that a substantial number of these bacteria were alive. With the exception of one day for the 4 mg/l unit, the plate counts for the nickel fed units were higher than the count obtained for the control.

There were practically no free swimming ciliated protozoa present in any of the nickel fed units; however, a few ciliated protozoa were almost always present in the

TABLE XII
EFFLUENT BACTERIAL COUNT
EXPERIMENTAL RUN 2

TIME	BACTERIAL COUNT, Number x 10^{-6}/ml					
	CONTROL*		2 mg/l	4 mg/l	7 mg/l	7 mg/l
Days	NO SLUG	SLUG	UNIT	UNIT	UNIT	UNIT
3	26.4		8.8	17.6	8.8	8.8
6	13.2		52.8	52.8	70.5	58.6
9	29.4		70.5	58.1	83.6	70.3
15	17.6		61.6	52.8	96.8	35.2
20	17.6		35.2	61.6	61.5	44.0
22	54.0		59.8	106.0	70.5	61.6
27	70.5		88.0	106.0	123.0	70.5
30	23.4		35.2	70.4	70.4	35.2
31	35.2	70.4	88.0	35.2	52.8	52.8
38	52.6	31.6	79.0	31.6	87.0	79.0

*One-half of the control received a 50 mg/l slug dose on the 30th day.

TABLE XIII
EFFLUENT STANDARD PLATE COUNT
EXPERIMENTAL RUN 2

TIME	PLATE COUNT, Number x 10^{-4}/ml					
	CONTROL*		2 mg/l	4 mg/l	7 mg/l	7 mg/l
Days	NO SLUG	SLUG	UNIT	UNIT	UNIT	UNIT
13	3.07		36.1	--	45.6	72.6
22	11.0		757	118	418	920
30	138		643	157	173	--
31	307	456	3740	119	348	427
38	40.8	106.5	109	167	51.0	152

*One-half of the control received a 50 mg/l slug dose on the 30th day.

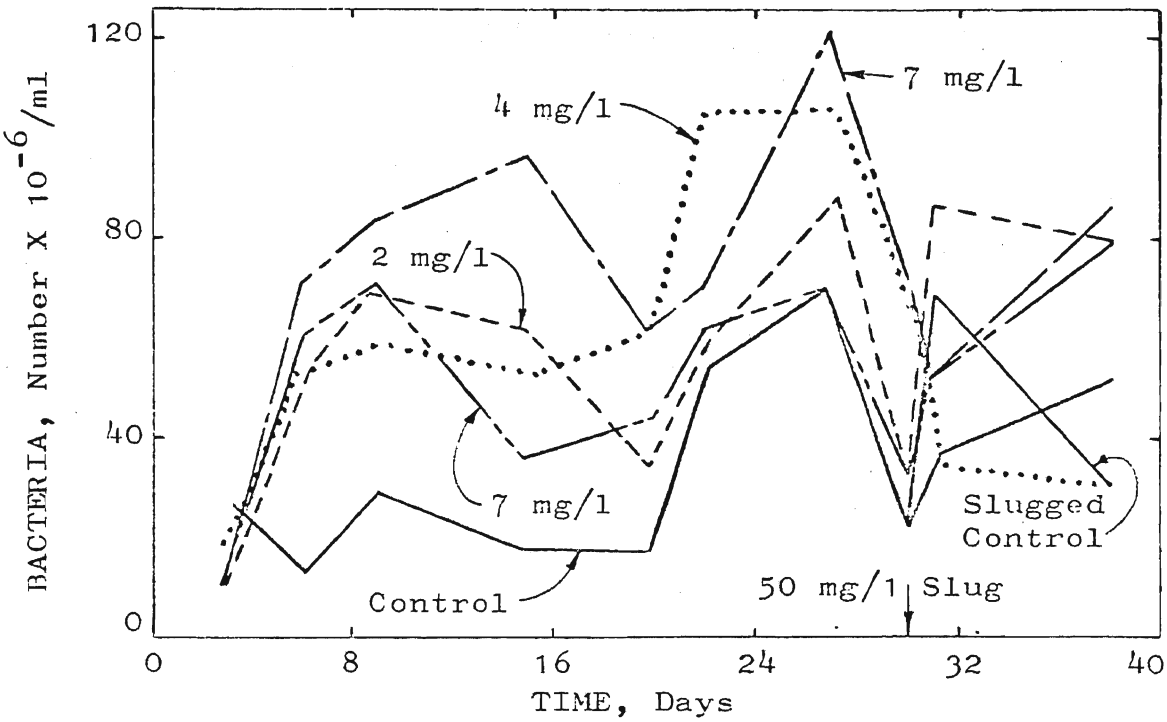


FIGURE 7. EFFLUENT BACTERIAL COUNT
EXPERIMENTAL RUN 2

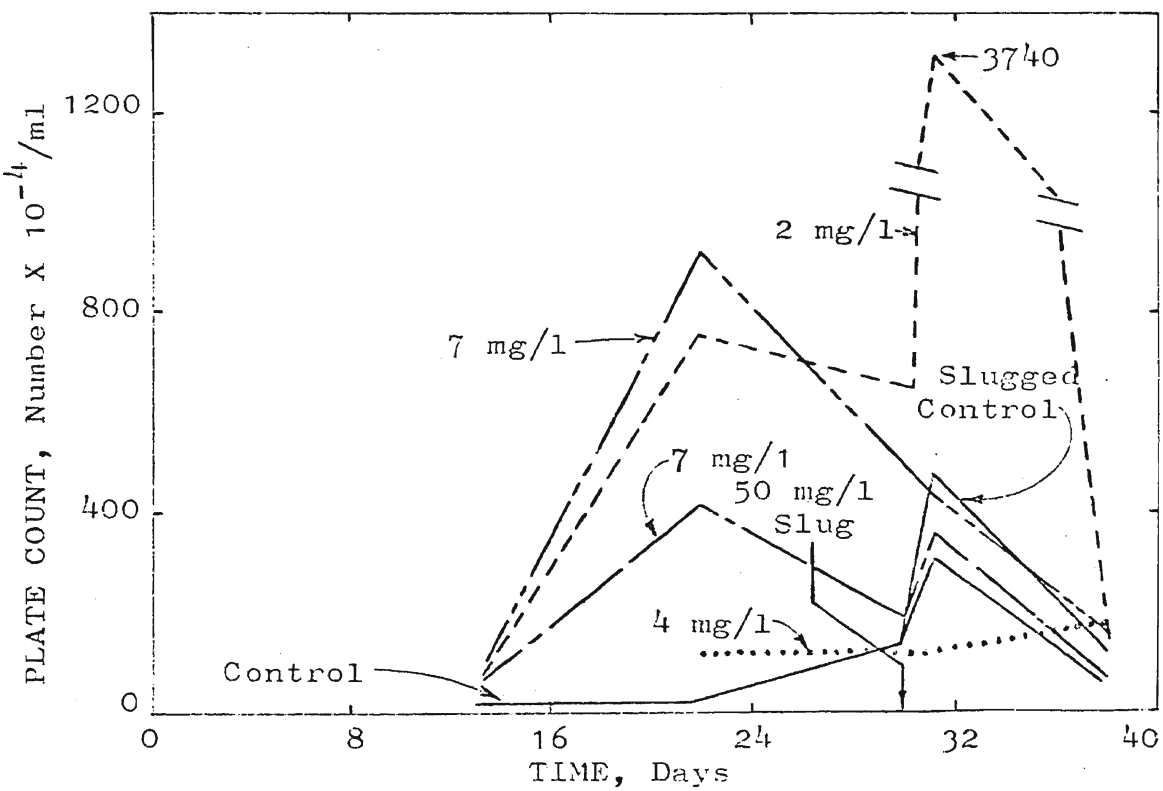


FIGURE 8. EFFLUENT STANDARD PLATE COUNT
EXPERIMENTAL RUN 2

control unit (Table XIV and Figure 9). This is in agreement with the report by Hill (5) which was previously mentioned, that paramecia, a type of free swimming ciliated protozoa, suffered greatly from the presence of nickel. On the contrary, as shown in Table XIV and Figure 9, a large number of stalked protozoa, primarily Vorticella and colonial forms were present in the mixed liquor of the nickel fed units and almost completely absent in the control unit. There were a number of rotifers (Table XV, Figure 9) present in the control and relatively few in the nickel fed units. The 2 and 4 mg/l nickel fed units did have some rotifers throughout the 40 day period, but in the two 7 mg/l units rotifers were practically nonexistent after the first 8 days. Characteristic was the rapid decrease in the number of rotifers and increase in the number of stalked protozoa in the control unit after the slug dose of 50 mg/l had been applied.

Four Warburg respirometer studies were made during the run to measure the oxygen uptake of the units after they had been exposed to nickel for increasing time intervals. The uptake of the control sludge was also determined at the same time. Sludge samples were withdrawn just prior to the feeding of the units. No additional source of substrate was provided. The Warburg respirometer data are given in Table XVI and plotted in Figure 10. On the 14th day increasing nickel doses progressively decreased the oxygen uptake.

TABLE XIV
FREE SWIMMING AND STALKED PROTOZOA COUNT
EXPERIMENTAL RUN 2

TIME	PROTOZOAN COUNT, Number/ml											
	CONTROL* NO SLUG		CONTROL* SLUG		2 mg/l UNIT		4 mg/l UNIT		7 mg/l UNIT		7 mg/l UNIT	
Days	FREE SWIM.	STALKED	FREE SWIM.	STALKED	FREE SWIM.	STALKED	FREE SWIM.	STALKED	FREE SWIM.	STALKED	FREE SWIM.	STALKED
1	44	160			44	102	116	189	29	145	174	29
3	101	87			0	72	0	15	15	29	15	72
6	116	87			0	43	0	0	0	44	0	58
8	116	0			0	73	15	29	0	29	0	15
10	131	15			0	58	0	29	0	0	0	0
17	15	44			15	638	217	203	15	377	15	580
20	29	15			15	667	29	246	15	435	0	290
22	29	58			0	667	0	435	0	406	0	435
24	44	29			0	770	15	520	15	540	0	650
27	131	15			0	784	0	769	29	624	0	595
30	15	15			0	783	0	754	0	304	58	464
31	0	29	0	0	0	898	15	798	0	145	0	623
35	0	0	0	29	0	626	0	450	0	232	0	435
40	29	0	189	507	203	420	0	407	116	304	73	623

*One-half of the control received a 50 mg/l slug dose on the 30th day.

TABLE XV
ROTIFER COUNT
EXPERIMENTAL RUN 2

TIME	ROTIFER COUNT, Number/ml					
	CONTROL*		2 mg/l	4 mg/l	7 mg/l	7 mg/l
Days	NO SLUG	SLUG	UNIT	UNIT	UNIT	UNIT
1	160		190	362	260	101
3	290		280	145	159	130
6	174		43	73	174	15
8	160		174	73	0	15
10	160		131	72	0	15
17	304		87	0	0	0
20	392		58	72	0	0
22	435		87	0	0	0
24	450		44	44	0	0
27	348		87	0	0	0
30	667		15	15	0	0
31	797	435	29	0	0	0
35	348	15	0	0	0	0
40	406	15	29	0	0	0

*One-half of the control received a 50 mg/l slug dose on the 30th day.

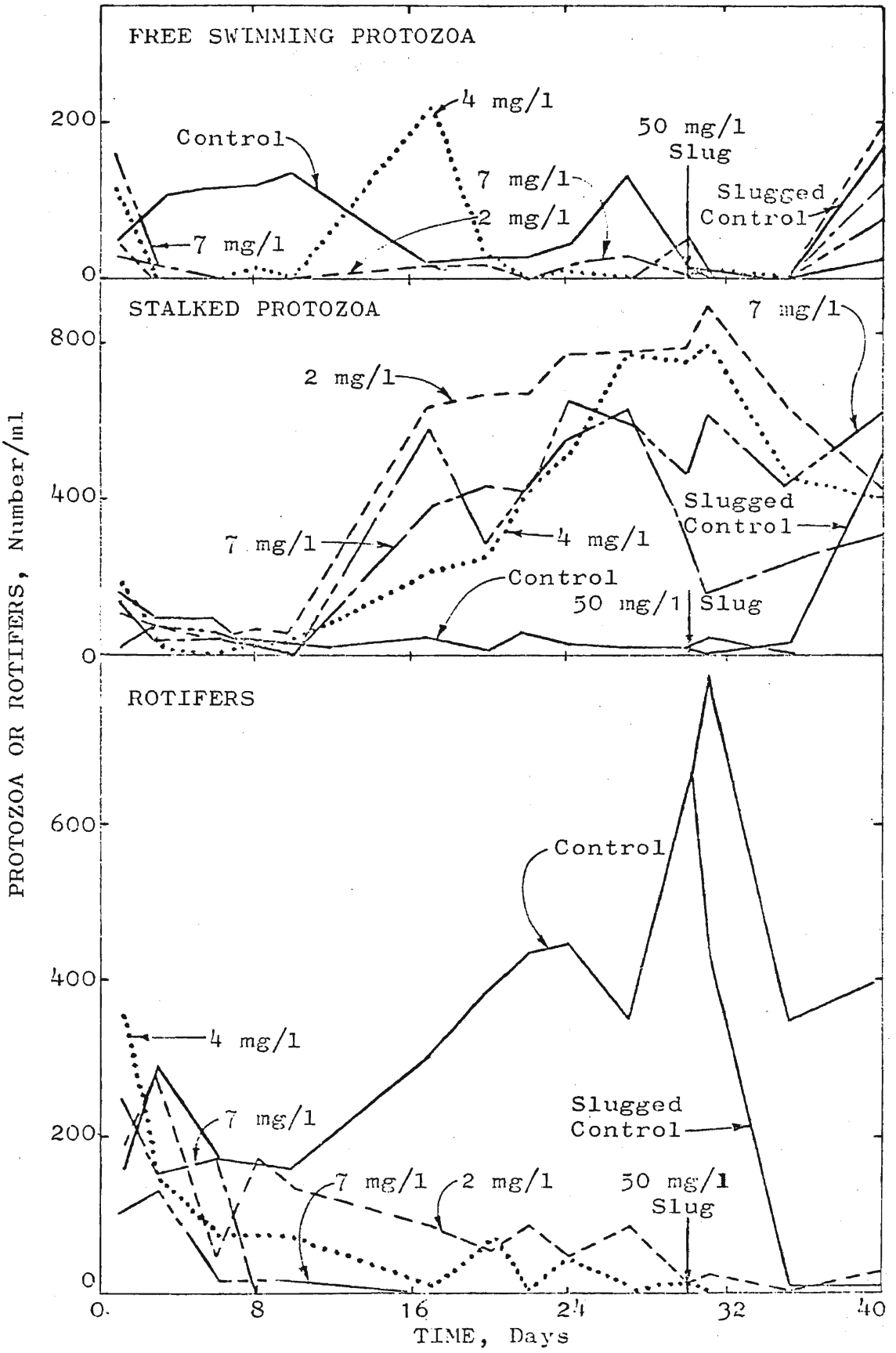


FIGURE 9. PROTOZOAN AND ROTIFER COUNT
EXPERIMENTAL RUN 2

TABLE XVI
OXYGEN UPTAKE OF ACTIVATED SLUDGE
EXPERIMENTAL RUN 2

TIME	CONTROL UNIT	2 mg/l UNIT	4 mg/l UNIT	7 mg/l UNIT	7 mg/l UNIT	SEWAGE
Hours	OXYGEN UPTAKE, mg/l					
ACTIVATED SLUDGE ON THE 14TH DAY OF OPERATION						
0	0	0	0	0	0	0
0.25	0	0	0.6	0	0.3	1.5
0.5	0.6	1.2	2.7	0.6	1.2	2.4
1	1.5	1.2	1.8	1.2	1.2	6.2
2	3.1	3.6	5.0	2.7	3.0	15.2
3.25	5.3	6.0	5.5	4.2	4.5	28.7
4.25	7.1	8.1	10.0	5.8	6.9	37.2
6	11.5	13.8	14.8	9.7	10.2	57.7
7	15.6	16.8	16.4	11.8	13.2	72.1
8	16.8	18.0	17.0	12.7	14.1	85.7
9.5	20.9	22.5	20.0	16.0	16.2	94.2
20.5	51.8	50.7	45.7	37.8	38.4	116
28	67.2	64.0	57.4	49.0	50.4	122
34.5	82.1	77.5	70.9	60.0	60.9	128
46	109	100	87.9	78.0	79.0	142
48	115	105	91.0	82.0	83.8	146
ACTIVATED SLUDGE ON THE 29TH DAY OF OPERATION						
0	0	0	0	0	0	0
0.25	0.3	0.3	0.9	0	0	0
0.5	0.3	0.3	0.9	0.3	0	0.9
1	0.3	1.2	0.9	0.6	0.6	3.5
2	3.7	1.8	2.5	1.8	1.2	5.5
3	5.0	3.3	4.3	3.9	2.7	7.0
4	6.5	5.4	6.5	6.0	4.8	8.5
6.5	10.7	9.3	12.0	11.2	8.4	10.5
8	13.1	11.4	15.7	13.9	11.7	12.1
9.75	16.3	14.1	19.1	16.9	15.0	12.9
19	30.0	26.1	35.8	30.8	30.6	16.4
25	38.7	33.0	41.6	40.5	40.2	17.3
32	46.2	40.2	46.6	51.0	51.0	19.1
43.5	61.8	54.4	56.1	69.2	68.4	23.1
48	65.2	57.3	59.0	75.5	74.8	23.7
51.5	70.9	62.6	63.2	82.1	81.0	25.8

TABLE XVI (Continued)
OXYGEN UPTAKE OF ACTIVATED SLUDGE
EXPERIMENTAL RUN 2

TIME	CONTROL* NO SLUG	CONTROL* SLUG	2 mg/l UNIT	4 mg/l UNIT	7 mg/l UNIT	7 mg/l UNIT	SEWAGE
Hours	OXYGEN UPTAKE, mg/l						
ACTIVATED SLUDGE ON THE 31ST DAY OF OPERATION							
0	0	0	0	0	0	0	0
0.25	0	0	0.6	0.6	0.3	0.3	0
0.5	0	0	0.9	1.2	0.6	0.3	1.8
1	0	0	0.9	1.2	0.6	0.3	3.0
2	0.9	0.3	1.5	1.8	0.6	0.6	5.7
3	3.7	1.5	2.5	3.3	2.4	1.8	11.3
4	4.0	2.1	3.7	3.6	2.4	2.6	13.1
5	5.6	2.7	4.3	4.5	2.7	2.6	14.1
6	5.6	2.7	4.6	4.8	3.3	2.6	14.1
9.5	11.2	6.4	9.2	8.2	6.3	6.1	17.0
14	13.1	7.5	9.8	8.8	6.6	6.4	17.0
19	22.4	12.6	17.0	15.1	12.3	12.0	20.3
22.5	26.2	14.7	20.3	17.2	13.5	12.9	20.3
32.5	36.8	22.2	26.8	24.8	19.8	19.6	23.9
39	47.5	29.7	36.7	33.2	28.0	27.0	29.6
45	50.5	31.8	39.7	35.6	29.4	29.3	29.6
47	52.1	32.8	41.0	36.8	30.9	30.5	29.9
57	62.3	38.7	47.8	42.3	36.6	36.1	30.2
ACTIVATED SLUDGE ON THE 34TH DAY OF OPERATION							
0	0	0	0	0	0	0	0
0.25	0	0	0	0	0	0	0
0.5	0.6	0.9	1.5	0.9	0.6	0	1.2
1	0.6	0.9	1.5	0.9	0.6	0	3.6
2	1.2	1.4	1.5	1.2	1.2	0.3	9.0
3	2.1	2.4	2.5	1.8	2.1	1.2	14.3
3.75	2.5	3.3	3.4	2.7	3.0	2.0	19.1
10	9.6	11.4	8.3	7.5	10.2	8.5	44.8
14.25	14.7	16.5	11.7	11.5	15.0	13.8	53.2
22.5	22.7	25.2	18.2	18.5	24.3	21.4	64.6
26.75	26.8	30.5	22.2	22.1	29.7	26.1	71.0
36	34.6	39.5	25.9	26.9	39.0	33.7	82.2
40.5	39.9	47.4	31.8	32.6	44.4	40.5	91.9
46.5	46.1	54.6	36.7	38.4	49.2	47.2	101
49.5	48.4	56.7	37.0	39.6	50.7	49.0	106
60.5	56.4	72.3	43.5	49.6	57.7	57.5	121
68	58.7	81.2	46.0	52.8	61.8	59.8	128
77	65.3	91.1	50.2	57.5	65.5	61.3	134
86	69.8	102	54.6	64.9	70.8	65.7	138

*One-half of the control received a 50 mg/l slug dose on the 30th day.

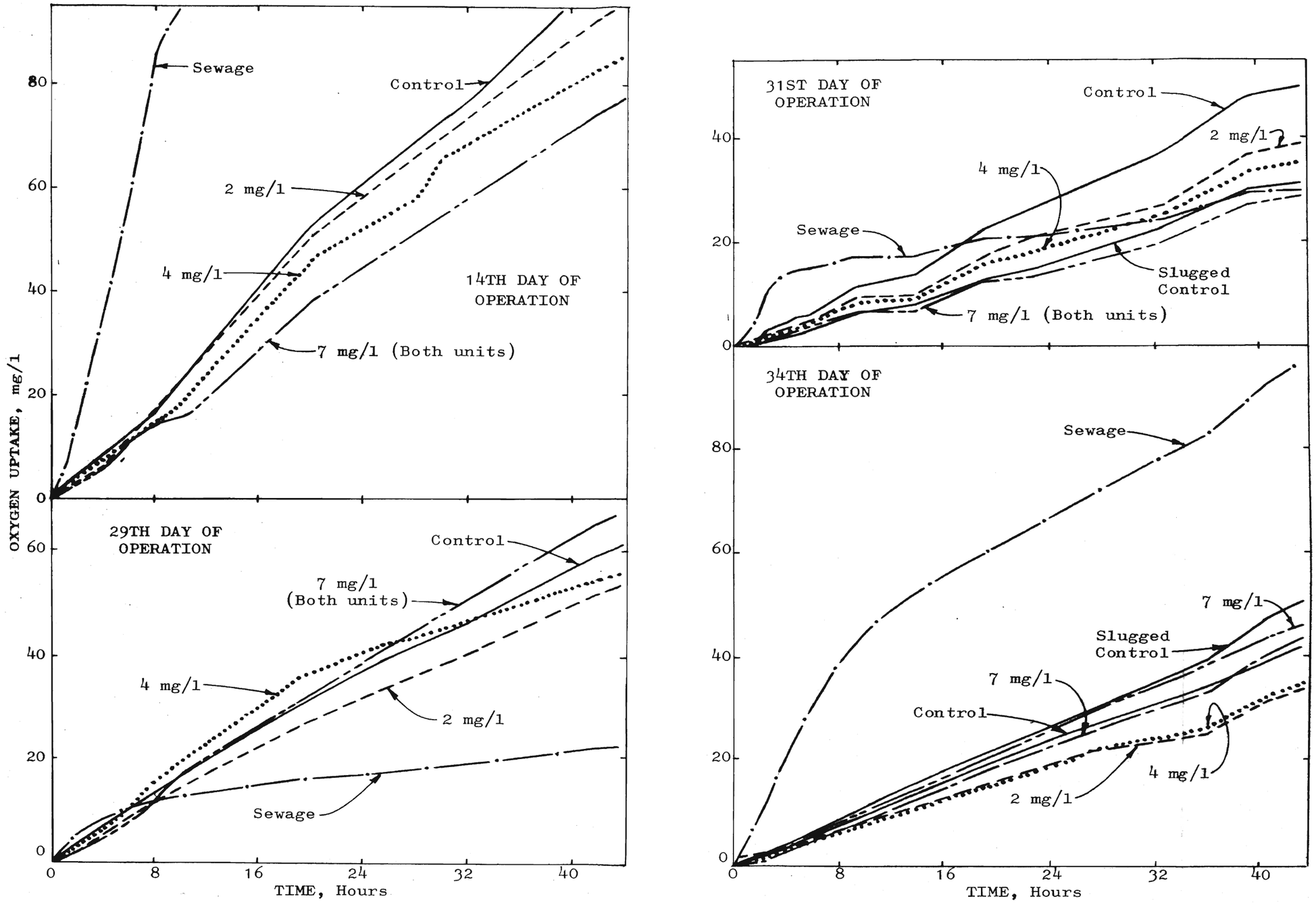


FIGURE 10. OXYGEN UPTAKE OF ACTIVATED SLUDGE
EXPERIMENTAL RUN 2

However, on the 29th day, the day before the slug dose was applied, the oxygen uptakes of the three units receiving the higher doses of nickel were about the same as the control for about 24 hours, but after that time the two 7 mg/l units showed more oxygen uptake than the control, while the 4 mg/l unit dropped below the uptake of the control. The oxygen uptake of the 2 mg/l nickel fed unit remained lower than the control throughout the run. On the 31st day, one day after the slug dose had been introduced, all units were affected. The oxygen uptake of the control was greater than that of the nickel fed units, and the uptake of the test units decreased as the concentration of the daily constant dose to which they had been exposed increased. The uptake of the slugged control was lower than that of the slugged 2 and 4 mg/l units. On the 34th day, 4 days after the nickel slug had been applied, the oxygen uptakes of the units returned to the same relative position as on the 29th day, with the oxygen uptake of the slugged control higher than those of all the other units after about 40 hours.

The nickel balances are shown in Table XVII and in Figure 11. The bottom portion of the graphs in Figure 11 represents the amount of metal in the sludge, determined from grab samples. The upper portion is the cumulative total amount of nickel lost from the systems in the effluent. Most of the nickel in all the units was accounted for.

TABLE XVII
NICKEL BALANCE
EXPERIMENTAL RUN 2

TIME Days	NICKEL ADDED mg	NICKEL IN SLUDGE mg	NICKEL IN EFF. mg	IMBAL-ANCE mg	IMBAL-ANCE %	NICKEL ADDED mg	NICKEL IN SLUDGE mg	NICKEL IN EFF. mg	IMBAL-ANCE mg	IMBAL-ANCE %
	2 mg/1 UNIT					4 mg/1 UNIT				
4	5.5	7.7	0	+ 2.2	+ 40	7.5	6.2	2.3	+ 1.0	+ 13
11	19.5	9.6	10.0	+ 0.1	+ 1	35.5	17.0	17.7	- 0.8	- 2
18	33.5	19.1	18.3	+ 3.9	+ 12	63.5	35.4	33.0	+ 4.9	+ 8
21	39.5	19.7	21.3	+ 1.5	+ 4	75.5	26.2	38.4	- 10.9	- 14
25	47.5	18.1	24.9	- 4.5	- 9	91.5	40.9	48.2	- 2.4	- 3
28	53.5	19.1	27.8	- 6.6	- 12	103.5	42.9	53.3	- 7.3	- 7
38	105.5	30.2	59.6	- 15.7	- 15	157.5	63.8	90.6	- 3.1	- 2
	7 mg/1 UNIT					7 mg/1 UNIT				
4	7.5	7.7	0	+ 0.2	+ 3	7.5	8.3	0	+ 0.8	+ 11
11	56.5	19.0	23.6	- 13.9	- 25	56.5	16.5	18.5	- 21.5	- 38
18	105.5	49.6	47.9	- 8.0	- 8	105.5	46.5	45.0	- 14	- 13
21	126.5	48.6	58.3	- 19.6	- 15	126.5	50.3	52.7	- 23.5	- 19
25	154.5	61.0	76.4	- 17.1	- 11	154.5	63.2	70.8	- 20.5	- 13
28	175.5	61.0	88.3	- 26.2	- 15	175.5	61.0	81.2	- 33.3	- 19
38	232.5	82.0	137.0	- 13.5	- 6	232.5	89.6	131.0	- 11.9	- 5

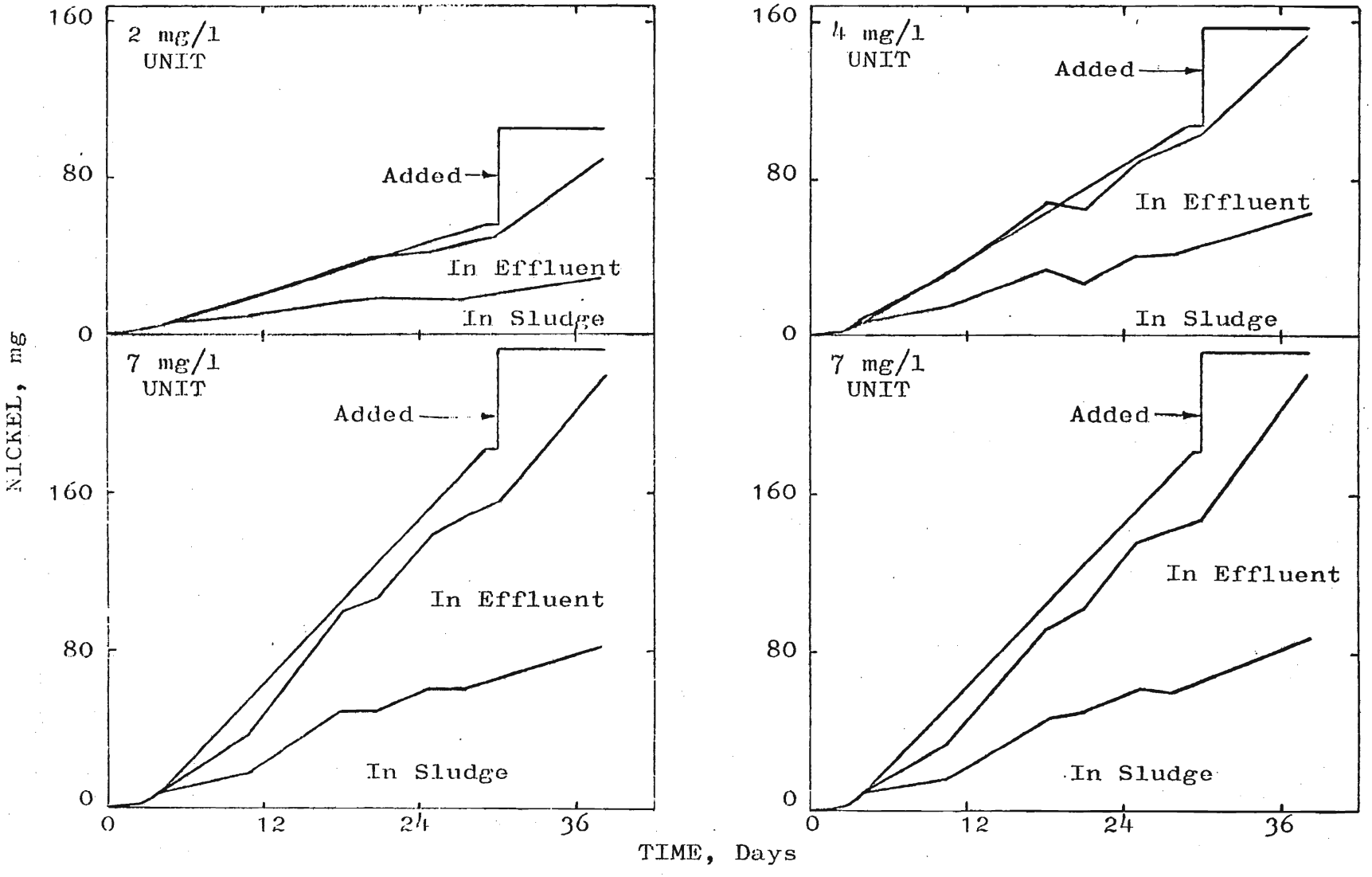


FIGURE 11. NICKEL BALANCE
EXPERIMENTAL RUN 2

Until the slug dose had been applied on the 30th day, the amount of nickel lost in the effluent was approximately the same as the amount accumulated in each system. Following the slug dose, however, nickel was discharged rapidly in the effluent, and the amount in the sludge increased only slightly. This would indicate that activated sludge would be exposed to the high nickel concentrations for a rather short period of time, especially in continuous flow systems. This is significant and would explain the ability of activated sludge to recover from slug doses.

Only 61 percent of the 25 mg of nickel added to the slugged control was accounted for. On the 38th day 5.9 mg was found in the sludge, and 9.3 mg was found to have been discharged in the effluent. These values, as well as those determined in the other four units, point out that substantial amounts of the slug were released in the effluents.

C. EXPERIMENTAL RUN 3

Run 3 was performed utilizing the same parameters as were used in Run 2. The nickel doses employed are shown in Table XVIII. Constant daily doses of 1, 4, 7, and 10 mg/l were applied to four of the units; on the 27th day, these units and one-half of the control were subjected to a slug dose of 50 mg/l. The concentration range of 1 to 10 mg/l was selected on the basis of the previous two runs. A 2 mg/l daily dose was shown in Runs 1 and 2 to increase the

TABLE XVIII
NICKEL FEED SCHEDULE
EXPERIMENTAL RUN 3

TIME	CONTROL* NO SLUG	CONTROL* SLUG	1 mg/l UNIT	4 mg/l UNIT	7 mg/l UNIT	10 mg/l UNIT
Days	NICKEL ADDED, mg/l					
0		0	0.5	0.5	0.5	0.5
1			1.0	1.0	1.0	1.0
2				2.0	2.0	2.0
3				4.0	4.0	4.0
4					7.0	7.0
5						10.0
26		0	1.0	4.0	7.0	10.0
27	0	50	50	50	50	50
28		0	0	0	0	0
40	0	0	0	0	0	0

*One-half of the control received a 50 mg/l slug dose on the 27th day.

effluent suspended solids and COD and to affect the microbial population. The 1 mg/l dose in this run was chosen in order to determine a possible toxicity threshold range, and the 10 mg/l dose was selected in an attempt to cause the breakdown of one of the units. The 4 mg/l dose was applied in both Runs 1 and 2, and the 7 mg/l dose was also used for two duplicate units maintained in Run 2.

The data obtained from effluent and filtered effluent COD determinations are shown in Table XIX and Figure 12. The effluent COD of the control was well below the corresponding values of the nickel fed units, as it was in the other runs; however, the COD's of the duplicating units were less than those in Runs 1 and 2. The filtered effluent COD values of the nickel fed units were again considerably smaller than the unfiltered values, but both values for the control were about the same. The maximum filtered effluent COD in the nickel fed units after the systems had been stabilized and before the addition of the slug dose was 48 mg/l, while the maximum in the control was 37 mg/l. The filtered effluent COD's increased somewhat after the addition of the slug on the 27th day, and the unfiltered effluent values were affected to a greater extent. Compared to the control, the 1 mg/l unit was affected much faster but also recovered more rapidly.

The total effluent and mixed liquor suspended solids data are shown in Table XX and Figure 13. For the same

TABLE XIX
EFFLUENT AND FILTERED EFFLUENT CHEMICAL OXYGEN DEMAND
EXPERIMENTAL RUN 3

TIME	COD, mg/l											
	CONTROL* NO SLUG		CONTROL* SLUG		1 mg/l UNIT		4 mg/l UNIT		7 mg/l UNIT		10 mg/l UNIT	
Days	EFF.	FILT. EFF.	EFF.	FILT. EFF.	EFF.	FILT. EFF.	EFF.	FILT. EFF.	EFF.	FILT. EFF.	EFF.	FILT. EFF.
2	36	28			32	57	24	44	32	48	16	44
9	22	26			61	31	48	35	79	48	92	44
15	34	30			73	30	90	43	98	43	64	43
21	17	22			52	30	61	30	70	30	56	35
23	34	22			77	30	77	34	73	39	86	43
27	--	21			54	33	54	37	54	41	99	37
28	45	37	61	29	114	33	90	41	77	41	106	45
33	28	24	121	44	73	36	81	40	85	44	125	40
36	18	27	72	63	36	50	68	54	95	63	108	68

*One-half of the control received a 50 mg/l slug dose on the 27th day.

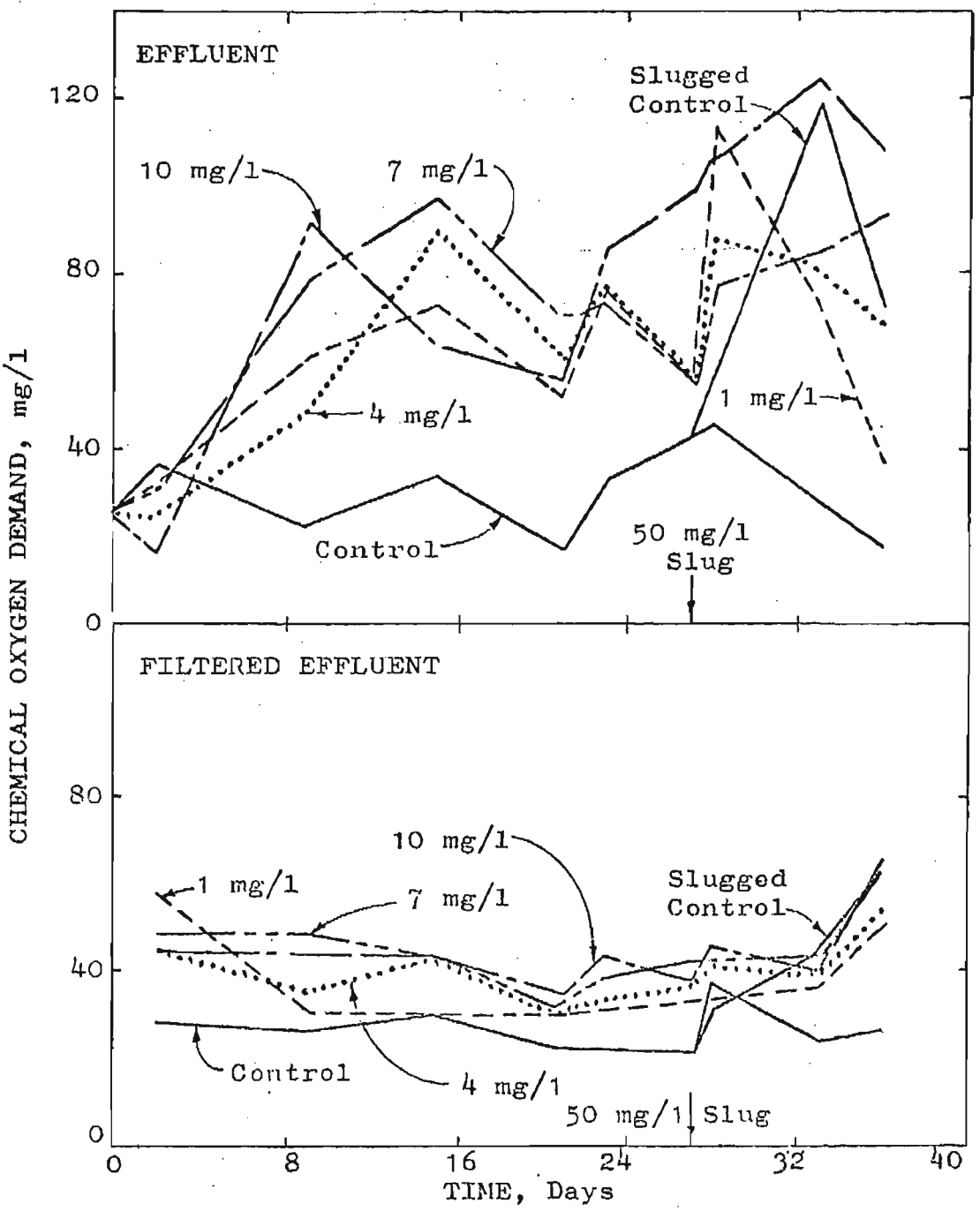


FIGURE 12. EFFLUENT AND FILTERED EFFLUENT CHEMICAL OXYGEN DEMAND EXPERIMENTAL RUN 3

TABLE XX
TOTAL EFFLUENT AND MIXED LIQUOR SUSPENDED SOLIDS
EXPERIMENTAL RUN 3

TIME	SUSPENDED SOLIDS, mg/l											
	CONTROL* NO SLUG		CONTROL* SLUG		1 mg/l UNIT		4 mg/l UNIT		7 mg/l UNIT		10 mg/l UNIT	
Days	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR	EFF.	MIXED LIQUOR
0	--	1840			--	1755	--	1770	--	1865	--	1805
2	23	1905			20	1790	10	1765	13	1965	10	--
9	7	2215			67	1895	47	2160	53	2260	43	2305
15	30	2040			50	1985	73	2120	70	2150	33	1605
20	--	1975			33	2030	37	2010	40	2070	23	1625
23	37	2210			73	1995	70	1945	70	1960	73	2360
27	--	2605	--	2670	47	2040	37	2025	13	2045	87	2245
28	13	--	50	--	83	--	70	--	30	--	87	--
32	--	2305	77	1890	23	1965	53	2160	27	2380	93	2160
36	13	1895	23	1760	10	2000	37	2095	40	2290	50	2145

*One-half of the control received a 50 mg/l slug dose on the 27th day.

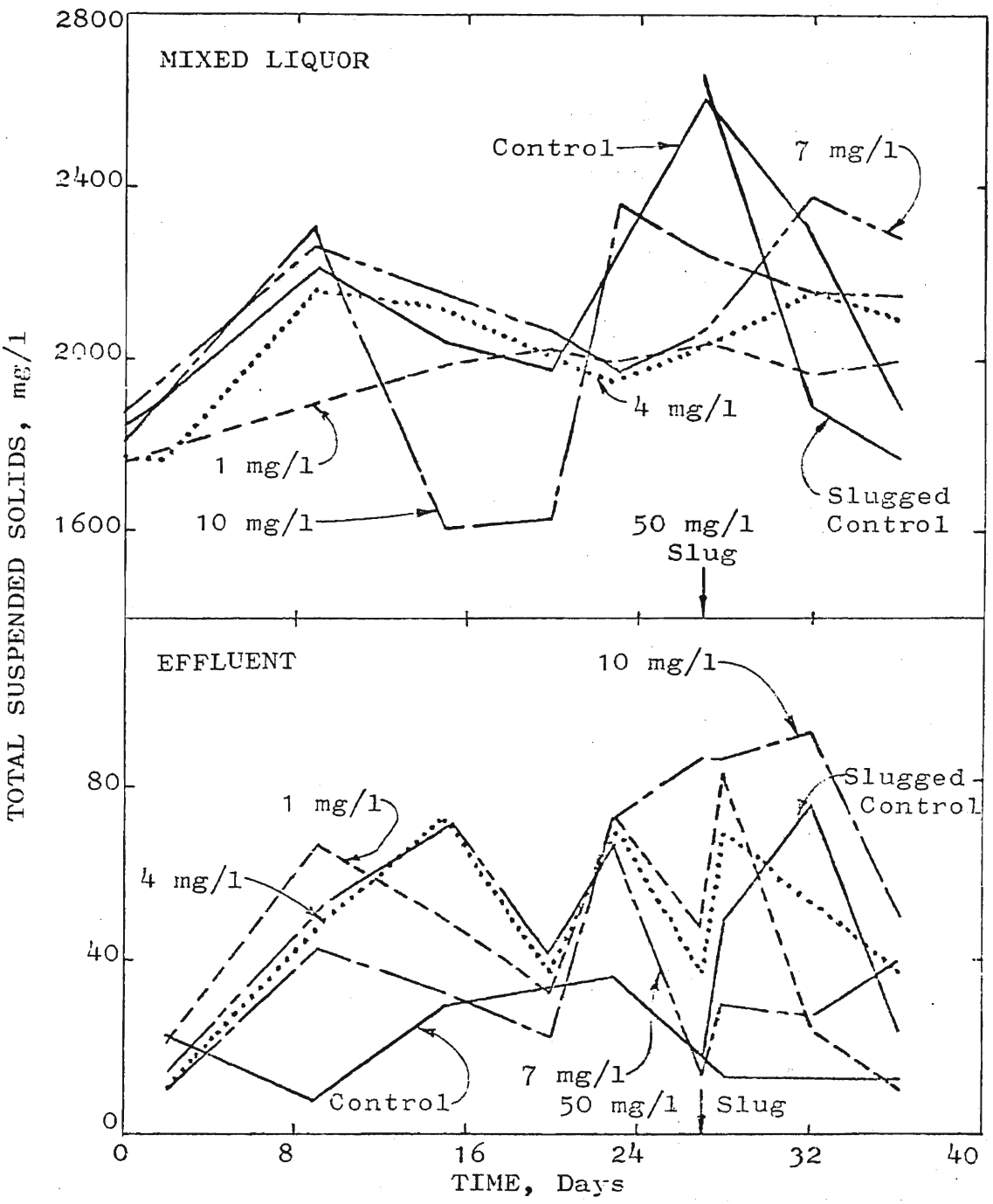


FIGURE 13. TOTAL EFFLUENT AND MIXED LIQUOR SUSPENDED SOLIDS EXPERIMENTAL RUN 3

units, the peaks of the effluent suspended solids curves corresponded to the peaks of the effluent COD curves, again showing the close relationship between effluent suspended solids and COD. The 1 mg/l nickel fed unit was definitely affected because of the high COD and suspended solids values determined in its effluent. As with the COD's, the effluent suspended values of the nickel fed units were also lower than the values obtained for the units fed the same nickel concentrations in Run 2.

The mixed liquor suspended solids remained fairly constant throughout the run with few exceptions (Table XX). The settled sewage strength (Table XXI) was also fairly uniform throughout the 40 day period. The sewage suspended solids were considerably higher than in Run 2. The volatile suspended solids ranged from 56 to 70 percent as shown in Table XXII, with the lower values occurring toward the end of the run.

Microscopic bacterial counts performed on the effluents are presented in Table XXIII and plotted in Figure 14. With the exception of the unit fed 1 mg/l, the nickel fed units had considerably higher counts than the control. The 1 mg/l unit also had a count which was higher than the control after the 21st day of operation. Standard plate counts (Table XXIV) indicated that, with a few exceptions, there were more live bacteria in the effluents of the nickel fed units than in the control. Following the addition of the

TABLE XXI
SETTLED SEWAGE CHARACTERISTICS
EXPERIMENTAL RUN 3

TIME PERIOD Days		SEWAGE CHARACTERISTICS, mg/l		
		CHEMICAL OXYGEN DEMAND		TOTAL SUSPENDED SOLIDS
FROM	TO	SEWAGE	MEMBRANE FILTERED SEWAGE	
0	1	249	--	55
2	9	303	101	117
10	15	241	92	97
16	22	200	91	53
23	29	232	86	117
30	40	265	118	90

TABLE XXII
VOLATILE SUSPENDED SOLIDS
EXPERIMENTAL RUN 3

TIME Days	VOLATILE SOLIDS, %					
	CONTROL* NO SLUG	CONTROL* SLUG	1 mg/l UNIT	4 mg/l UNIT	7 mg/l UNIT	10 mg/l UNIT
2	63		65	64	65	--
9	65		65	65	67	70
20	64		62	64	61	62
36	59	58	57	56	56	55

*One-half of the control received a 50 mg/l slug dose on the 27th day.

TABLE XXIII
EFFLUENT BACTERIAL COUNT
EXPERIMENTAL RUN 3

TIME	BACTERIAL COUNT, Number x 10^{-6}/ml					
	CONTROL*		1 mg/l	4 mg/l	7 mg/l	10 mg/l
Days	NO SLUG	SLUG	UNIT	UNIT	UNIT	UNIT
10	68.4		63.2	103	89.6	158
21	31.6		23.7	55.3	47.4	47.4
23	19.8		47.4	79.0	111	42.0
27	31.6		31.6	34.8	79.0	31.6
28	23.7	55.3	83.0	103	95.0	71.0
36	15.8	63.2	36.8	52.6	68.4	68.4

*One-half of the control received a 50 mg/l slug dose on the 27th day.

TABLE XXIV
EFFLUENT STANDARD PLATE COUNT
EXPERIMENTAL RUN 3

TIME	PLATE COUNT, Number x 10^{-4}/ml					
	CONTROL*		1 mg/l	4 mg/l	7 mg/l	10 mg/l
Days	NO SLUG	SLUG	UNIT	UNIT	UNIT	UNIT
10	5.5		78.5	57.8	101	275
23	6.9		24.1	16.4	24.8	67.3
28	9.3	6.1	9.0	8.1	16.4	25.0
36	27.6	91.8	1090	493	344	157

*One-half of the control received a 50 mg/l slug dose on the 27th day.

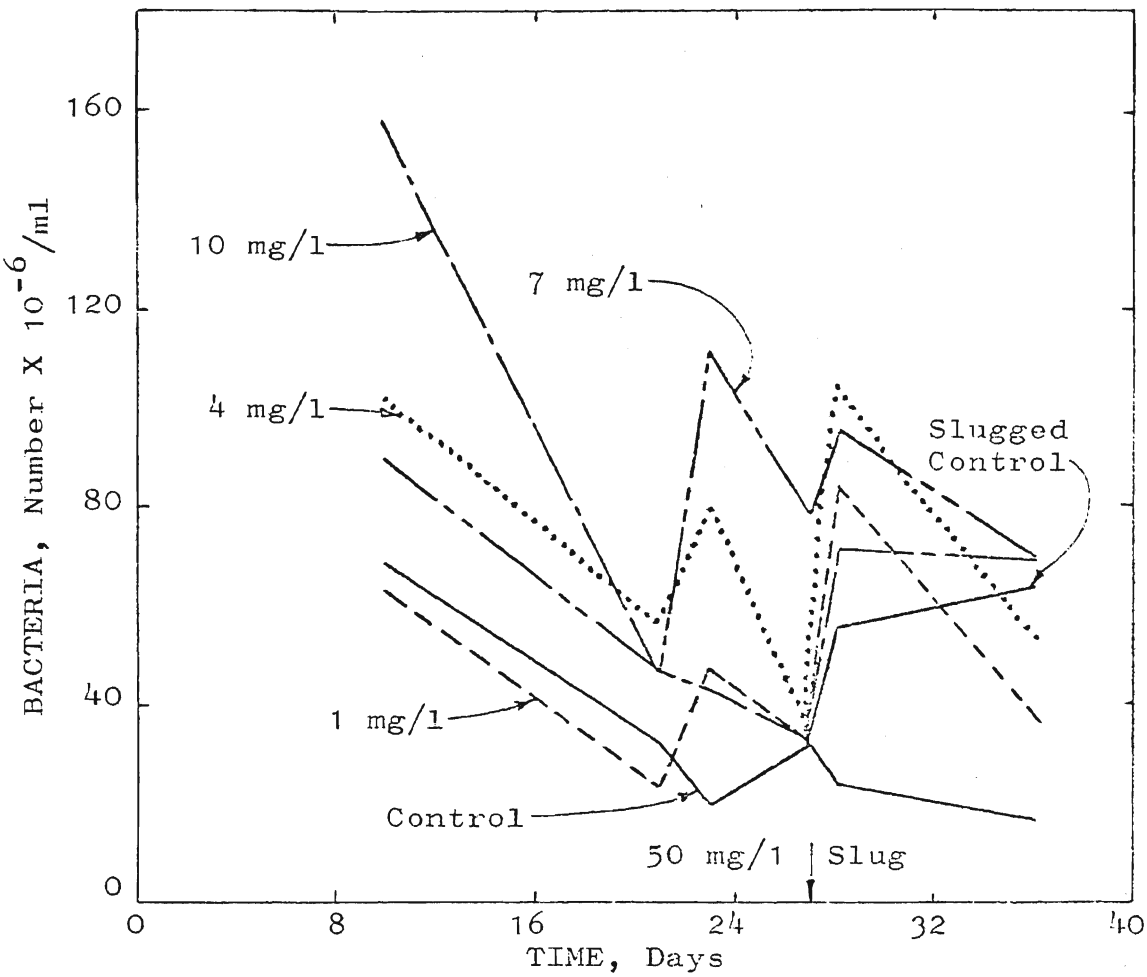


FIGURE 14. EFFLUENT BACTERIAL COUNT
EXPERIMENTAL RUN 3

slug dose, the effluent bacterial count increased (Figure 14), while the number of live bacteria decreased (Table XXIV). However, 9 days after the slug dose had been added the total effluent bacterial count decreased, and the number of live bacteria increased. It should be pointed out that nickel was not added to any of the units during this period.

Free swimming ciliated protozoa (Table XXV and Figure 15) were present in the control practically throughout the run, and their number was large in several cases. Usually more were present than found in the control of Run 2. However, as in Run 2, few free swimming ciliated protozoa were observed in the nickel fed units. These animals seemed to be strongly affected by the presence of nickel. On the contrary there were generally more stalked protozoa in the nickel fed units than in the control (Table XXV and Figure 15). After the addition of the slug dose the numbers of protozoa in all slugged units were greater than in the corresponding units of Runs 1 and 2.

With the exception of the 1 mg/l unit, there were less rotifers present in the nickel fed units than in the control (Table XXVI and Figure 15). The rotifer count in the 4 mg/l unit compared very favorably with the counts for the units receiving the same nickel dose in Runs 1 and 2. It should be noted that the population of stalked protozoa increased, while the population of rotifers decreased after the addition of the slug dose.

TABLE XXV
FREE SWIMMING AND STALKED PROTOZOA COUNT
EXPERIMENTAL RUN 3

TIME	PROTOZOAN COUNT, Number/ml											
	CONTROL* NO SLUG		CONTROL* SLUG		1 mg/l UNIT		4 mg/l UNIT		7 mg/l UNIT		10 mg/l UNIT	
Days	FREE SWIM.	STALKED	FREE SWIM.	STALKED	FREE SWIM.	STALKED	FREE SWIM.	STALKED	FREE SWIM.	STALKED	FREE SWIM.	STALKED
0	0	30			0	60	0	30	0	30	0	30
3	75	15			30	15	0	0	0	15	15	15
9	120	60			90	75	15	330	45	240	0	60
16	165	180			15	60	0	195	15	675	135	225
22	840	195			0	165	45	585	45	795	60	270
24	795	180			15	135	0	870	45	705	0	360
27	90	135			30	315	0	465	30	435	0	435
28	195	345	0	120	0	615	0	345	0	255	0	180
32	735	195	0	0	0	285	15	360	45	1200	0	1080
33	480	180	0	0	0	555	15	570	0	1390	15	1450
37	15	15	60	1440	0	615	30	1370	0	450	0	30
40	30	0	30	1560	45	495	0	510	0	210	0	120

*One-half of the control received a 50 mg/l slug dose on the 27th day.

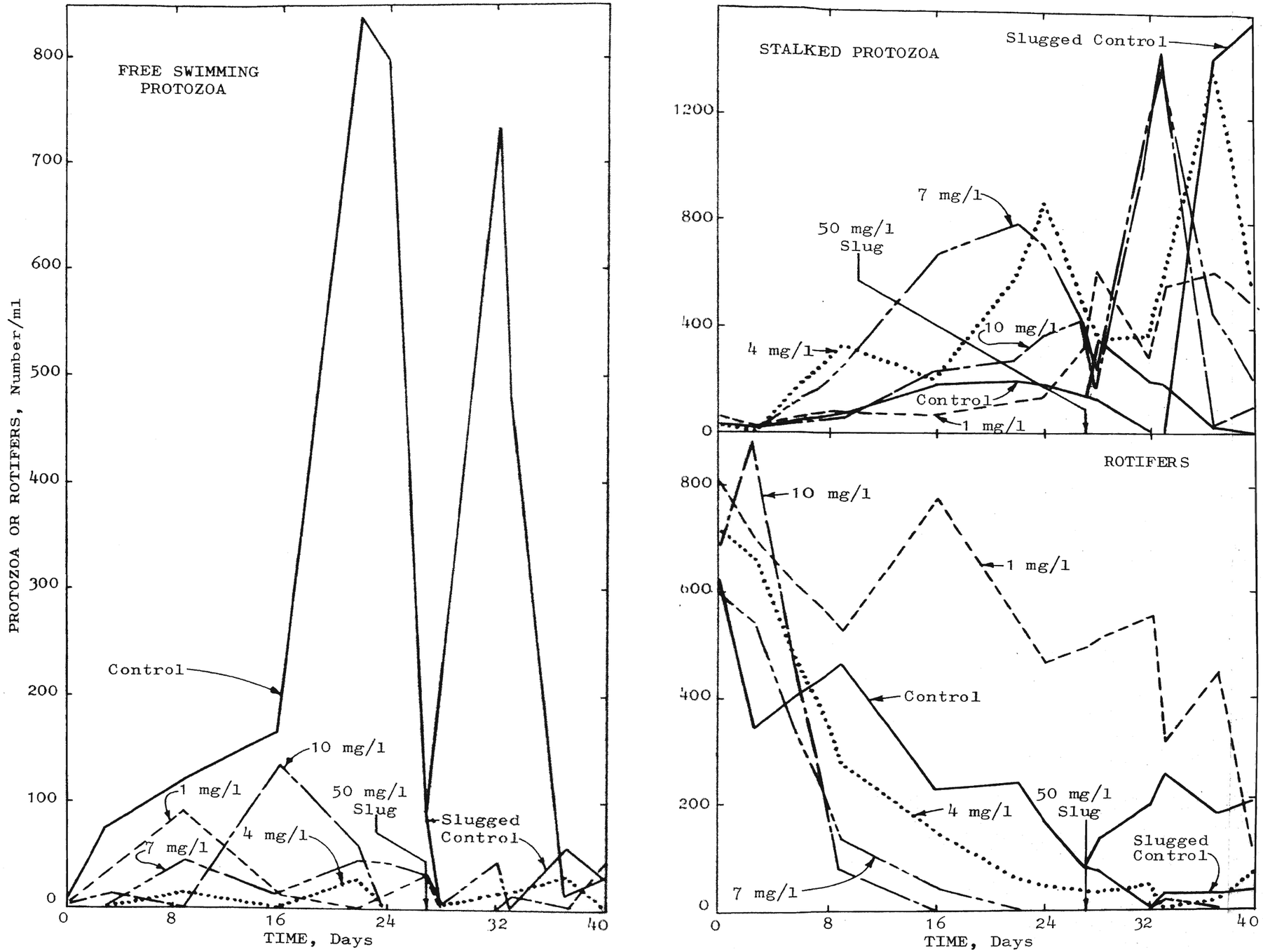


FIGURE 15. PROTOZOAN AND ROTIFER COUNT
EXPERIMENTAL RUN 3

TABLE XXVI
ROTIFER COUNT
EXPERIMENTAL RUN 3

TIME	ROTIFER COUNT, Number/ml					
	CONTROL*		1 mg/l	4 mg/l	7 mg/l	10 mg/l
Days	NO SLUG	SLUG	UNIT	UNIT	UNIT	UNIT
0	645		810	720	600	660
3	345		690	660	540	885
9	465		525	270	135	75
16	225		780	150	45	0
22	240		540	60	0	0
24	165		465	45	0	0
27	75		495	75	0	0
28	135	75	510	30	30	0
32	195	0	555	45	0	0
33	255	30	315	0	15	0
37	180	30	450	15	0	0
40	210	45	90	75	0	0

*One-half of the control received a 50 mg/l slug dose on the 27th day.

The oxygen uptake of the activated sludges was evaluated on the 11th, 23rd, 30th, and 33rd day of operation and the data obtained are shown in Table XXVII and Figure 16. On the 11th day of operation, the uptake of the 1 mg/l unit was slightly higher, and the uptake of the 10 mg/l unit was much higher than the control. The 4 and 7 mg/l units were below the control and very close together. On the 23rd day, the uptake of the 1 mg/l unit was still higher than the control, but the 4, 7, and 10 mg/l nickel fed units were close together and were less than the control. On the 30th day, 3 days after the addition of the slug dose, the 1 mg/l unit uptake was still higher than the control, while the other units showed lower uptakes and were positioned below the control in the order of 7, 4, and 10 mg/l, with the slugged control slightly below the 7 mg/l unit. On the 33rd day of operation, the units were about the same as the control except the 1 mg/l nickel fed unit which lagged slightly behind.

The nickel balances for Run 3 are shown in Table XXVIII and Figure 17. As in Run 2, the bottom portion of the graphs represents the amount of nickel in the sludge, while the top portion is the cumulative total amount of nickel lost in the effluent. Until the slug dose had been applied, the amounts accumulated and the amounts lost in the effluent remained approximately equal; however, following the slug dose more nickel was lost in the effluent than was accumulated in the sludge. Effluent nickel determinations

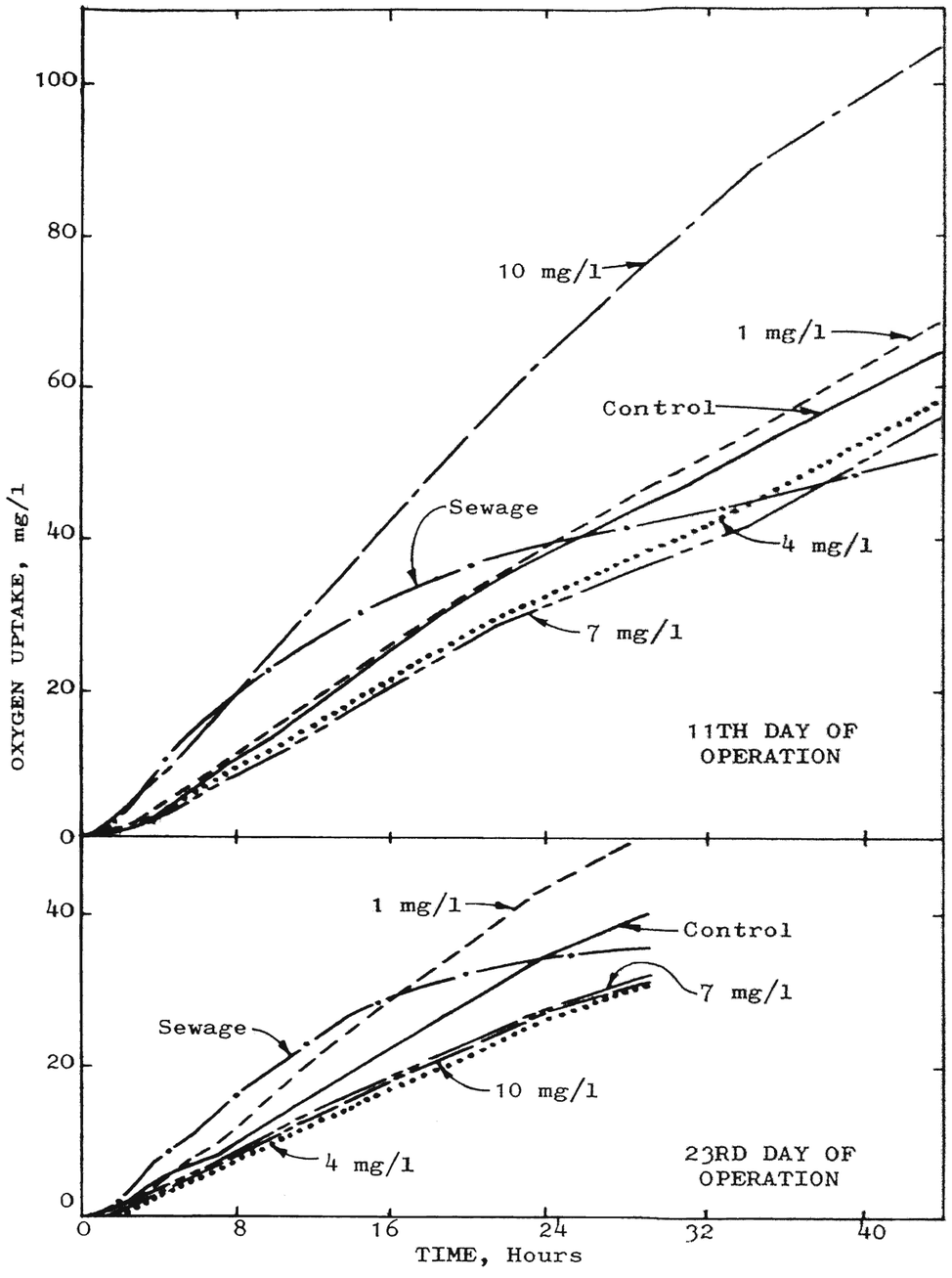
TABLE XXVII
OXYGEN UPTAKE OF ACTIVATED SLUDGE
EXPERIMENTAL RUN 3

TIME	CONTROL UNIT	1 mg/l UNIT	4 mg/l UNIT	7 mg/l UNIT	10 mg/l UNIT	SEWAGE
Hours	OXYGEN UPTAKE, mg/l					
ACTIVATED SLUDGE REMOVED ON 11TH DAY OF OPERATION						
0	0	0	0	0	0	0
0.5	0	0	0	0	0	0
2	1.2	1.4	1.7	1.8	3.3	4.4
3	2.5	3.1	2.7	2.1	7.1	7.1
4	4.0	4.8	3.9	3.9	8.6	11.0
5	5.9	6.6	5.3	4.6	11.3	14.6
7.5	10.3	11.4	9.5	8.9	18.7	19.7
9	12.8	13.4	11.1	11.0	23.0	23.0
10	14.0	15.1	12.0	11.6	25.1	24.2
21	35.0	34.8	29.0	28.4	56.3	37.8
31	47.1	49.7	40.7	38.8	81.7	43.5
34	51.8	53.7	44.3	41.9	88.8	45.0
44.5	65.5	69.7	58.5	56.8	106	52.2
46	68.4	72.3	60.7	59.0	112	52.2
50	74.9	79.2	67.1	65.7	120	56.1
60	84.6	91.5	77.7	76.5	133	60.3
67.5	94.9	104	87.7	88.0	147	63.2
72	96.2	107	90.5	89.5	150	66.8
81	107	120	100	102	163	70.1
ACTIVATED SLUDGE REMOVED ON 23RD DAY OF OPERATION						
0	0	0	0	0	0	0
0.5	0	0	0	0.6	0.3	0
1.75	0	0	0	0.6	0.3	0.9
3.5	4.1	3.4	2.4	3.3	2.5	6.8
5.5	6.8	7.1	4.7	5.2	4.3	11.0
7	8.4	9.8	6.7	7.3	6.4	14.3
11.5	15.2	19.7	12.5	13.1	12.5	22.4
23.5	33.7	42.8	25.9	26.6	26.6	34.3
29.25	40.3	50.9	30.6	31.7	30.6	35.9

TABLE XXVII (Continued)
OXYGEN UPTAKE OF ACTIVATED SLUDGE
EXPERIMENTAL RUN 3

TIME	CONTROL* NO SLUG	CONTROL* SLUG	1 mg/l UNIT	4 mg/l UNIT	7 mg/l UNIT	10 mg/l UNIT	SEWAGE
Hours	OXYGEN UPTAKE, mg/l						
ACTIVATED SLUDGE REMOVED ON 30TH DAY OF OPERATION							
0	0	0	0	0	0	0	0
1	0	2.0	2.2	1.5	2.1	0.6	2.6
2	2.5	2.5	4.7	3.3	3.3	1.5	6.3
4.75	8.1	7.1	12.8	7.3	7.4	4.4	19.1
7	12.1	9.4	19.0	10.4	10.4	6.3	24.3
8	15.0	11.1	21.7	12.2	11.6	7.4	26.4
10	19.0	14.6	26.4	15.3	14.4	9.8	30.7
12.5	22.1	16.3	31.4	18.0	18.0	11.6	33.6
22	40.5	30.8	56.3	31.1	33.4	22.3	44.3
26	44.4	34.6	64.0	34.5	37.4	25.1	45.8
36	60.0	48.8	76.6	45.8	52.0	34.9	51.9
48	78.0	66.8	87.1	59.6	69.8	43.6	58.8
61	94.2	83.2	97.7	71.2	85.4	54.6	65.2
70	104	93.7	105	79.5	97.3	--	72.2
ACTIVATED SLUDGE REMOVED ON 33RD DAY OF OPERATION							
0	0	0	0	0	0	0	0
0.5	0.6	0	0.2	1.0	0	0	0.5
2	1.5	2.5	3.9	2.8	3.3	1.2	7.8
4.5	5.6	7.4	8.6	7.0	9.2	6.2	21.7
7	10.3	13.1	13.1	12.0	13.1	11.3	27.6
9.5	15.6	18.9	17.3	17.4	18.3	17.0	32.8
18.5	32.1	40.9	30.9	33.6	32.4	35.5	47.0
22	36.5	45.7	34.3	40.0	37.3	41.5	49.3
25	43.1	50.9	39.5	47.6	45.0	51.0	53.7
31	50.6	56.3	46.5	55.9	52.1	59.4	57.2
33	54.3	59.1	49.5	60.5	56.6	64.0	59.2
43	70.2	69.5	59.6	77.6	70.7	80.6	64.7
46.5	76.6	73.1	63.0	87.9	75.4	85.6	67.4
53	83.3	79.8	69.4	98.5	83.5	91.2	72.9
56	86.2	84.0	72.6	102	87.2	92.5	75.7
69	101	97.5	82.1	111	102	100	87.3

*One-half of the control received a 50 mg/l slug dose on the 27th day.



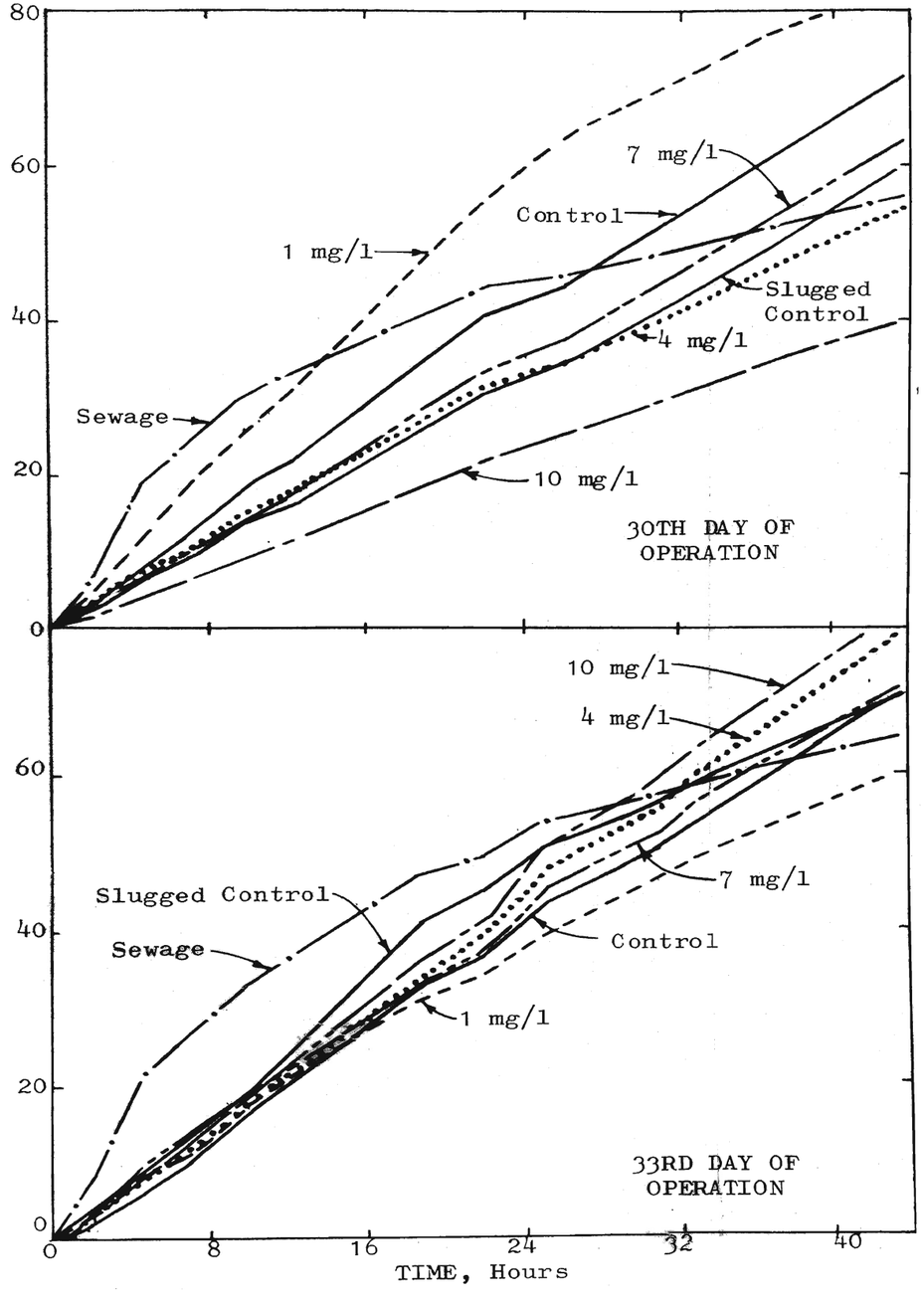


FIGURE 16. OXYGEN UPTAKE OF ACTIVATED SLUDGE
EXPERIMENTAL RUN 3

TABLE XXVIII
NICKEL BALANCE
EXPERIMENTAL RUN 3

TIME Days	NICKEL ADDED mg	NICKEL IN SLUDGE mg	NICKEL IN EFF. mg	IMBAL-ANCE mg	IMBAL-ANCE %	NICKEL ADDED mg	NICKEL IN SLUDGE mg	NICKEL IN EFF. mg	IMBAL-ANCE mg	IMBAL-ANCE %
	1 mg/l UNIT					4 mg/l UNIT				
4	3.5	2.6	1.8	+ 0.9	+ 26	7.5	5.0	1.8	- 0.7	- 9
11	10.5	2.0	5.7	- 2.8	- 27	35.5	21.3	11.6	- 2.6	- 7
15	14.5	11.7	6.8	+ 4.0	+ 28	51.5	32.7	17.2	- 1.6	- 3
20	19.5	5.9	12.4	- 1.2	- 6	71.5	39.6	34.1	+ 2.2	+ 3
27	26.5	14.0	21.5	+ 9.0	+ 34	99.5	29.0	53.0	- 17.5	- 18
28	--	--	22.7	--	--	--	--	55.9	--	--
29	76.5	--	28.2	--	--	149.5	--	69.2	--	--
34	76.5	35.1	48.0	+ 6.6	+ 9	149.5	50.0	89.0	- 10.5	- 7
	7 mg/l UNIT					10 mg/l UNIT				
4	7.5	5.5	1.8	- 0.2	- 3	7.5	4.5	1.8	- 1.2	- 16
11	56.5	42.7	14.8	+ 1.0	+ 2	74.5	59.3	24.8	+ 9.6	+ 13
15	84.5	52.5	24.5	- 7.5	- 9	114.5	59.2	36.7	- 18.6	- 16
20	119.5	80.6	48.6	+ 9.7	+ 8	164.5	80.6	64.7	- 19.2	- 12
27	168.5	76.0	78.7	- 13.8	- 8	234.5	98.5	101.8	- 34.2	- 15
28	--	--	83.1	--	--	--	--	108.0	--	--
29	218.5	--	89.3	--	--	284.5	--	115.8	--	--
34	218.5	107.0	113.4	+ 1.9	+ 1	284.5	103.0	146.7	- 34.8	- 12

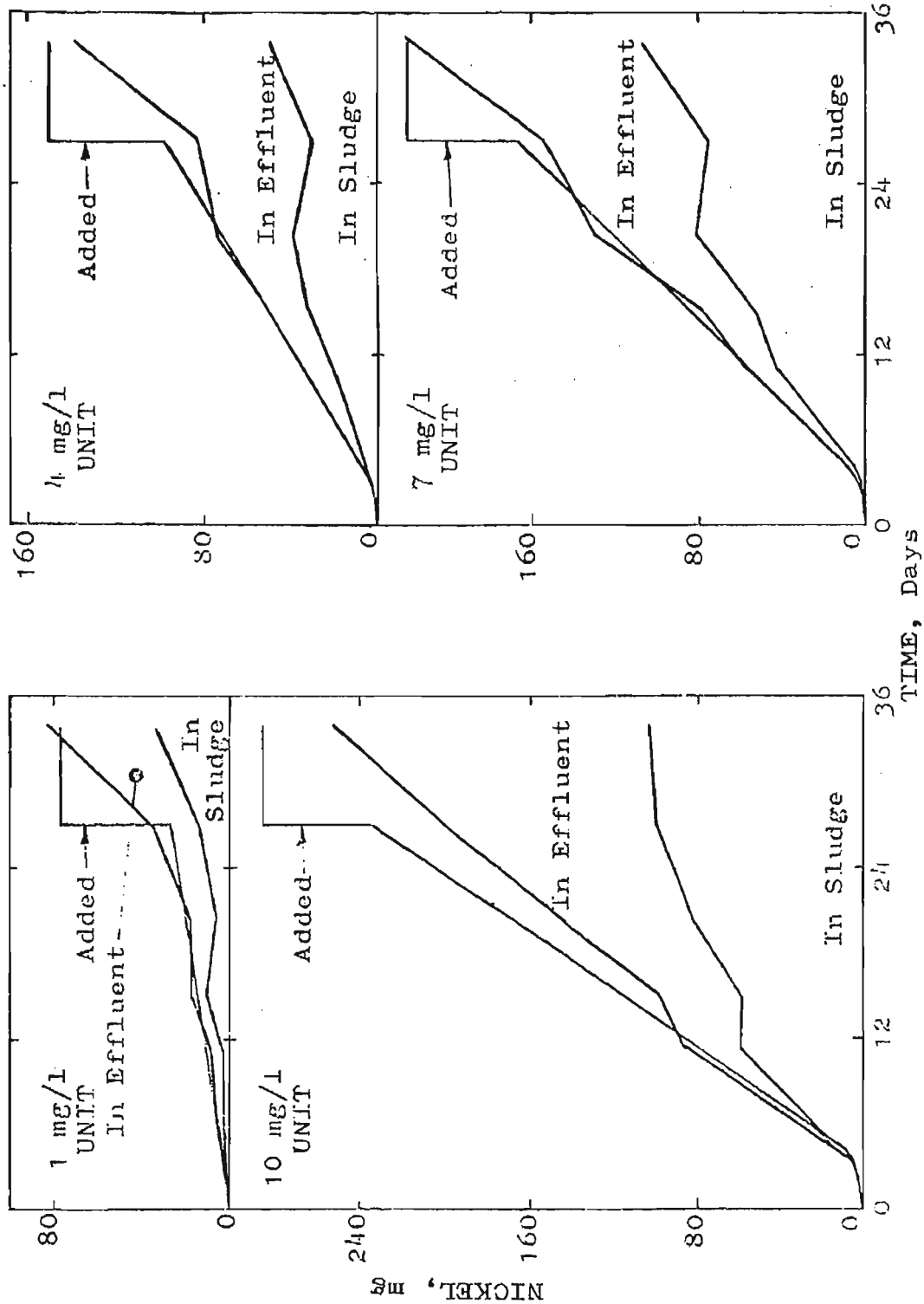


FIGURE 17. NICKEL BALANCE EXPERIMENTAL RUN 3

performed 24 hours after the addition of the slug dose showed a small amount of nickel present, and several days were required for it to be released in the effluent. Of the 25 mg of nickel applied to the slugged control on the 27th day, 1.1 mg was lost in the effluent after 24 hours of aeration; at the end of 6 days after the slug dose had been applied an additional 6.4 mg of nickel had been lost, giving a total of 7.5 mg released in the effluent. At the same time 10.1 mg of nickel were found in the sludge. This distribution is not in agreement with the results of Run 2 which indicates that more nickel was lost in the effluent than retained in the sludge. Approximately 70 percent of the nickel added to the slugged control was accounted for in this run, as compared to 61 percent accounted for in Run 2.

V. DISCUSSION

The idea for this study originated from reports that nickel fed activated sludge produced an effluent high in COD and turbidity, and that protozoa seemed to suffer from the presence of nickel. The study was developed in order to verify the reported increase in effluent COD and suspended solids, and attempt to relate it to the animal population present in nickel fed activated sludge. Nickel balances were also made to verify the reported limited affinity of activated sludge for nickel, and attempt to correlate it with the ability of sludge units to recover from slug doses.

In 1956, McKinney and Gram (4) demonstrated that the presence of protozoa in activated sludge was necessary in order to produce an effluent of good quality. Later on in his book, Microbiology for Sanitary Engineers, McKinney (15, p. 139) again pointed out the importance of protozoa in the sludge system noting that the predominance of particular types of protozoa followed a fixed pattern more closely than any other group of microorganisms. Some of the animal population dynamics curves presented by McKinney are reproduced in Figure 18.

Protozoa and rotifers can ingest solid matter, can feed upon bacteria, both dead and alive, and consequently help clarify the effluent. When a waste is first introduced

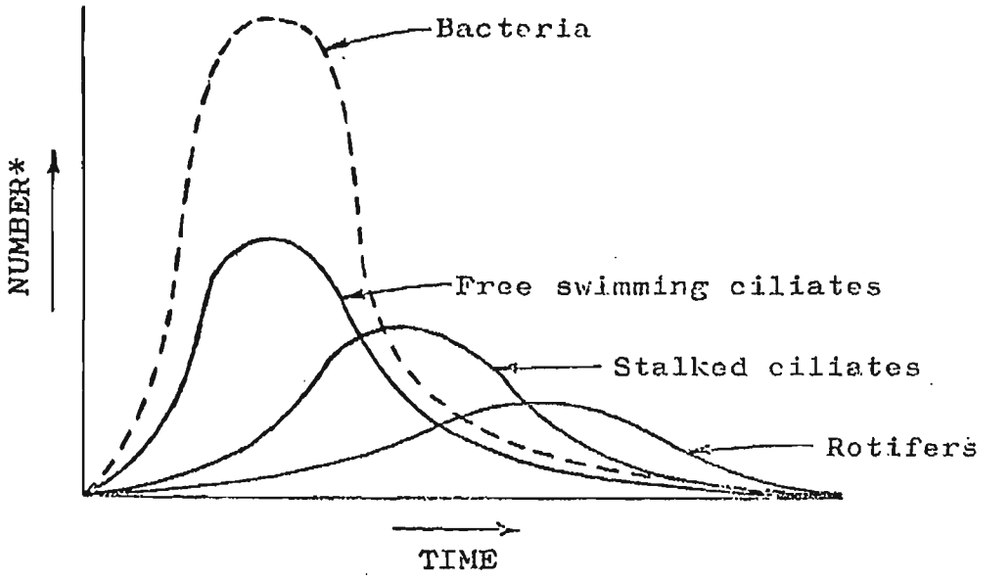


FIGURE 18. RELATIVE GROWTH OF PROTOZOA AND ROTIFERS [After McKinney (13, p. 140)]

*Number of microorganisms not to same scale, but expanded for clarity.

to an activated sludge unit, the soluble organic material and the solid organic matter which can readily be broken down by hydrolysis are plentiful and the bacteria begin to grow rapidly. As the number of bacteria increases, free swimming ciliated protozoa begin to grow because they use the bacteria for food. As long as the bacterial population is high, the free swimming protozoa population will also be high. Free swimming protozoa have very high energy requirements, and when the bacterial population decreases because of depletion of soluble organics and from being consumed they cannot obtain enough energy to survive. The stalked protozoa, which have lower energy requirements, then begin to predominate. Eventually, the system is so stable that even the stalked protozoa cannot survive and rotifers and other higher animals take over. These organisms have the ability to utilize the nonsoluble fraction of the dead bacteria, as well as other solid organic particles. Protozoa and/or rotifers should, therefore, be present in a well functioning activated sludge, with the predominating type depending upon the organic stability of the system.

Total suspended solids determinations and microscopic and standard plate counts of bacteria were made on samples of the effluents from the nickel fed activated sludge units and the control in order to attempt to correlate any increase in suspended solids to a greater number of bacteria present. Effluent and membrane filtered effluent COD

determinations were performed in order to find how much of the increased effluent COD from nickel fed activated sludge units was caused by suspended matter. This increase in organic suspended matter could then be related to a greater number of bacteria in the effluent.

All of the nickel concentrations studied (1-10 mg/l) caused an increase in total effluent suspended solids and COD. The effluent suspended solids and COD curves for the respective units had essentially the same shapes, and their peaks corresponded, indicating that there was a close relationship between effluent suspended solids and COD. After the addition of the 50 mg/l slug dose, the effluents from the nickel fed units (in both Runs 2 and 3) were not greatly affected; however, 6 days after the addition of the slug the effluent suspended solids and COD values of the slugged controls were higher than those of the units which had received constant daily nickel doses of 1, 2, and 4 mg/l. This supports the findings by Matthews (3) relative to the ability of acclimated activated sludge units to overcome the effects of slug doses.

The effluent COD and suspended solids values in Run 2 were generally higher than those in Runs 1 and 3. Heukelekian and Gellman (10) have reported that a decrease in sewage strength resulted in increased repression of oxygen uptake, and Matthews (3) attributed increased effluent COD's from batch activated sludge units to decreased sewage

strength. The strength of the settled sewage used in Run 2 was considerably less than the strength of the sewages employed in Runs 1 and 3, and this could account for the higher effluent COD and suspended solids values observed in Run 2.

The COD values of the effluent from the control unit in both Runs 2 and 3 were not much greater than the corresponding filtered effluent COD's, while in each case the effluents from the nickel fed units were much greater than the corresponding filtered effluent values. The COD values of the filtered effluents from all the nickel fed units remained relatively low during the runs, even after the 50 mg/l slug dose had been applied. Therefore, the major portion of the increased COD values observed in the nickel fed units was caused by organic matter in the effluent suspended solids, rather than by untreated soluble organics.

In Run 2, some high and erratic effluent COD and suspended solids values were found in the nickel fed units up until about the 8th day of operation, at which time the values began to level off. The 8 liter stock unit which was used for developing the sludge to be placed in the test activated sludge units was exposed to the sun and supported considerable algal growth. Diatoms were observed in measurable numbers on stained slides prepared from the effluents of the nickel fed units, but very few were seen

in the effluent from the control. This was not reflected in the COD of the filtered effluents. It is believed that the algae present in the stock activated sludge were destroyed by the nickel, and the dead cells caused the high effluent COD and suspended solids values.

The total suspended solids of the settled sewage fed to the activated sludge units in Run 2 (Table X) were less than the suspended solids of the effluents from the nickel fed units. This pointed out that something more than untreated sewage suspended solids was going through the systems. On the contrary, the sewage suspended solids in Run 3 (Table XXI) were generally slightly higher than the effluent suspended solids values obtained for the nickel fed units during that run. With the data obtained it is impossible to determine what part of the effluent suspended solids was due to sludge lost in the effluent and what part was due to unhydrolyzed sewage solids. The microorganisms in the nickel fed units were inhibited, as shown by the oxygen uptake curves. This inhibition could have decreased the efficiency of the organisms to break down the sewage solids, and allowed more solids to leave the nickel fed unit than were released from the control unit. The use of settled sewage as a substrate for feeding the laboratory activated sludge units enabled the experimental conditions to more closely simulate the conditions that would be encountered in actual field operation. Difficulty was

experienced, however, in securing sewage of uniform organic strength and suspended solids concentration. A completely soluble synthetic sewage of uniform strength would have eliminated this difficulty, but would have deviated further from actual conditions.

Microscopic bacterial counts (Figures 8 and 14) indicated that more bacteria were present in the effluents from the nickel fed units than from the controls. This held true throughout Run 2 until the addition of the slug dose at which time one value for the 4 mg/l unit was below that of the control. It was also true for Run 3, with the exception of the 1 mg/l unit during part of the run. The bacterial counts measured the total number of bacteria, both dead and alive, since differentiation was not possible by this method. Also, the use of a simple stain did not enable the determination of the bacterial types present; the use of more involved techniques, beyond the scope of this investigation, might have been able to detect a change in bacterial predomination in the nickel fed units as the runs progressed.

Standard plate counts, which measure only viable bacteria, indicated that there were more live bacteria in the effluents from the nickel fed units than from the control. An analysis of the standard plate count data (Tables XIII and XXIV) revealed that until the slug doses had been applied the plate counts of the nickel fed units

were in most cases several times that of the control. In both runs, 24 hours after the addition of the slug doses the ratio between the number of live bacteria in the effluent from the nickel fed units and from the control decreased, with the exception of the 2 mg/l unit in Run 2. This ratio increased again in 8 or 9 days in both runs. Also after 24 hours the plate counts of the slugged controls were not much different than those of the controls to which no nickel was added, but in both runs 8 or 9 days after the slug had been applied the counts were several times that of the controls. After the addition of the slug dose, the effluent total bacterial counts, by microscopic examination, of the nickel fed units were greater than found for the control unit, with the exception of the 4 mg/l unit in Run 2; standard plate counts indicated that fewer of these bacteria were alive 24 hours after the addition of the slug dose than were alive before the slug dose had been added and 8 or 9 days after its addition. This would indicate that the slug dose was detrimental to some bacteria.

There were few free swimming ciliated protozoa observed in either Runs 1 or 2, while they were present most of the time in the control of Run 3 and were numerous on several occasions. In Run 2, a few ciliated protozoa were almost always present in the control unit; however, only in isolated instances were any found in the nickel fed units. Generally a few more were found in the nickel fed units of

Run 3 than of Run 2, but many more were found in the control of Run 3 than were present in the control of Run 2.

In all three runs there were usually more stalked protozoa present in the nickel fed units than were found in the controls. From the 25th day to the end of Run 1 there were practically no stalked protozoa found in the control, while in Run 2 the population of stalked protozoa remained low in the control throughout the run. The number of stalked protozoa in the control of Run 3 was generally higher than in the other two runs, and the number in the slugged control of Run 3 was much greater than in the slugged control of Run 2. The stalked protozoa population in the slugged controls increased sharply in both Runs 2 and 3 sometime between the 6th and 10th day after the addition of the 50 mg/l dose.

At concentrations ranging from 2-10 mg/l, nickel had a detrimental effect upon the rotifer population in the sludge, while at a concentration of 1 mg/l it seemed to stimulate their growth. A small number was always found in the 2 mg/l units of Runs 1 and 2, and the population in the 4 mg/l units was very low after 22-25 days in all 3 runs. There were no rotifers seen in the 7 mg/l units in Runs 2 and 3 after 16-23 days of operation, and no rotifers were observed in the 10 mg/l unit in Run 3 after 10 days. The number of rotifers observed in the control in Run 3 was usually less than in Runs 1 and 2. This could be related

to the increased number of free swimming ciliated protozoa observed in Run 3. The settled sewage fed to the units in Run 2 was considerably weaker, as the run progressed, than that used in Run 3. The higher strength of sewage employed in Run 3 would tend to make a less stable system which would not be as encouraging to the growth of rotifers. A stronger sewage would result in more bacterial growth which, in turn, would result in a greater population of free swimming protozoa.

One day after the addition of the slug dose in Runs 2 and 3 the rotifer population in the slugged controls decreased, indicating extreme toxicity. However, the population in the 1 mg/l unit remained greater than that of the control for more than 10 days following the addition of the slug dose; this would indicate that the rotifer population might have become acclimated to the 1 mg/l constant daily nickel dose.

Rotifers were the predominating animals in the control units. The stalked and free swimming ciliated protozoa population in these units remained low during most parts of the runs. According to McKinney (15, p. 237), a regular activated sludge unit with a 4-6 hour detention time producing an effluent of 5-10 mg/l of BOD should have a relatively active population of stalked ciliated protozoa with a small number of rotifers or free swimming ciliated protozoa; and a unit with an effluent of 10-20 mg/l of

BOD should have a high and approximately equal number of free swimming ciliates and stalked protozoa. The total oxidation system with a detention time of approximately 24 hours produces an effluent of less than 5 mg/l of BOD and the system is so stable that only a few rotifers are visible as living animals.

The experimental units were fed one-half of their total volume each day (a detention time of 48 hours) with none of their sludge wasted, and could be, therefore, considered as total oxidation systems. This would explain the predominance of rotifers in the controls. This is further substantiated by the low effluent COD values of the controls which indicated a good environment for rotifers whose metabolic habits limit them to surroundings of low organic content (15, p. 51). With the exception of the 1 mg/l unit, the rotifer population decreased in all nickel fed units. This could have been caused by at least two reasons; either the toxicity of the nickel could have destroyed the rotifers, or the effluent could have deteriorated to such an extent that there was too much organic matter present to encourage their growth. The 1 mg/l nickel fed unit supported a sizeable number of rotifers 13 days after the addition of the slug dose, while the number in the slugged control decreased within one day following the addition of the slug dose. As seen from Figures 12 and 13, both the 1 mg/l unit and the slugged control showed a sharp increase in effluent COD

and suspended solids after one day following the slug dose. After this period, the slugged control continued to increase in both values, while the 1 mg/l unit began to recover rapidly. It could be that the effluent quality of the 1 mg/l unit recovered so rapidly that the rotifers were able to survive, while the longer recovery time required by the slugged control discouraged the rotifer population. This would support the supposition that the increased organic content of the effluents caused a decrease in the rotifer population.

The predominance of the stalked forms of protozoa found in the nickel fed units was not in line with what was expected from the protozoa and rotifer population dynamics presented in Figure 18. The stalked protozoa were expected to be the predominant type only in situations where there was not a high enough bacteria and suspended solids concentration in the activated sludge to sustain a population of free swimming ciliated protozoa. It should be noted that the nickel fed units had a significant concentration of mixed liquor suspended solids and that volatile solids determinations indicated that a large part of the mixed liquor suspended solids were organic. This, and the observation that much of the suspended matter in the effluents of the nickel fed units was organic, as verified by the difference between the effluent and membrane filtered effluent COD values, indicates that the environment of the

nickel fed units should have been very readily amenable to the growth of large numbers of free swimming ciliated protozoa. The suitability of this environment for the growth of free swimming ciliated protozoa was further demonstrated by the increased number of dead and viable bacteria found in the effluents of the nickel fed units. The environment would not appear to be favorable to the observed growth of stalked forms of protozoa. In addition, few rotifers were expected in the unstable nickel fed activated sludge units and this expectation was verified in this study.

The population of stalked protozoa in the nickel fed units was low at the beginning of each run and several days were required for their number to increase above that of the control. There were no indications that these higher stalked protozoa populations were preceeded by a large number of free swimming ciliated protozoa as would be anticipated on the basis of the population dynamics suggested by McKinney (Figure 18).

The presence of measureable numbers of active stalked protozoa, as found in this study, was contrary to what was anticipated from the report by Hill (5). However, Hill found that nickel mostly affected the units which were fed sewage containing 6 or 10 mg/l nickel. Since his sludge units were fed three times each day, the total daily nickel fed was much greater than that used in the present study,

and the detention time was considerably less than the 48 hours employed in the present investigation. Because of the different relative stabilities of the two systems, the populations of the protozoa of different types and of rotifers would be, therefore, expected to differ in the two experiments.

The free swimming ciliated protozoa are very efficient in removing excess bacteria and other suspended matter because they are able to move around, and due to their high energy requirements require more substrate. Stalked protozoa, on the other hand, are able to survive with less substrate and, being less active than free swimming protozoa, ordinarily consume less. Therefore, it appears that the almost complete absence of free swimming protozoa in the nickel fed units was responsible for the increased effluent COD and suspended solids observed for these units.

Oxygen uptake studies were performed to provide a measure of the condition of the activated sludge units. The oxygen uptakes (Figures 10 and 16) were relatively low, since sludge samples were taken prior to feeding and an additional substrate was not added. Consequently, the uptakes gave primarily a measure of the activity of the microorganisms while undergoing endogenous respiration; but also measured respiration while removing residual organic matter which had not been utilized in the aeration period preceeding the Warburg studies and while the sludge

was still in the units. The latter depended to a large extent on the strength of the sewage that had been used for the daily feeding of the units.

In all cases, with the constant daily nickel doses used and the slug doses employed the units continued to function, as was demonstrated by their oxygen uptakes. In most cases, the oxygen uptakes during the addition of the constant daily doses and a short time after the addition of the slugs were lower than that of the control. Several instances occurred, however, where the oxygen uptake of some of the units was greater than that of the control.

On the 29th day of operation in Run 2 the duplicate 7 mg/l units had higher uptakes than any of the other units. On that day the 7 mg/l units had higher effluent COD and effluent suspended solids values and a lower stalked protozoa count than either the 2 or 4 mg/l units. On the 31st day of Run 2, 24 hours after the addition of the slug dose, the oxygen uptakes of the duplicate 7 mg/l units were much lower than that of the control and slightly less than those of the 2 and 4 mg/l units. The slugged control uptake was below that of the 2 and 4 mg/l units, while its effluent COD and suspended solids increased more than those of the 2 and 4 mg/l units, as shown in Figures 5 and 6. This supported the findings by Matthews (3) that a system can react more efficiently to a slug dose of nickel, if acclimated to small doses. On the 11th day of Run 3 the

oxygen uptake of the 10 mg/l unit was substantially above that of all the other units. The greater oxygen uptakes of the units receiving higher doses of nickel appeared to be characteristic of certain time periods for both Runs 2 and 3. This could be due to the presence of bacteria of a particular type which were capable of acclimating to nickel and able to metabolize readily in its presence. At the time the oxygen uptakes were measured the effluents from the duplicate 7 mg/l units in Run 2 and from the 10 mg/l unit in Run 3 had been deteriorating for several days. This build up of organic matter in the units would be available, in addition to the daily sewage feed, to any bacterial type which could acclimate to the presence of nickel, and the residual substrate would allow the micro-organisms in the nickel fed units to exhibit a greater oxygen uptake than those in the control units where the only substrate was the daily addition of sewage.

In both Runs 2 and 3, nickel applied as a slug dose was discharged at a faster rate in the effluent than was accumulated in the sludge (Figures 11 and 17). The 7 mg/l unit in Run 3 accumulated more nickel in the sludge than the duplicate 7 mg/l units in Run 2. This could be due to the higher mixed liquor suspended solids and the higher activity of the sludges in Run 3 as shown by the oxygen uptake curves (Figures 10 and 16).

On the 13th day of Run 2 a nickel determination was performed on the filtrate of the mixed liquor from one of the 7 mg/l nickel fed units. Results indicated that only about 15 percent of the total nickel found in the mixed liquor was present in the washed filtrate. This would indicate that most of the nickel was incorporated in the sludge and could not be removed by ordinary washing with distilled water.

McDermott, et al. (1) reported that activated sludge removed 25-27 percent of the nickel applied, and Stones (7) reported a 30 percent removal by activated sludge. The data presented here indicate that about 50 percent of the nickel was removed by the sludges. McDermott, et al. used a continuous flow system with a continuous sludge return and a continuous sludge waste of about 5 percent. They had more sludge growth than was possible in the present study, since they had a system with an aeration period of 6 hours. No sludge was wasted in the present study. The sludge acclimated to the nickel concentrations could have accumulated more nickel than the sludge in the continuous flow system which had more sludge growth and new cells.

A nickel determination performed 24 hours after the addition of the slug dose in Run 3 showed a small amount of nickel being present (Table XXVIII) indicating that much of the slug dose had accumulated in the sludge during the first 24 hours. However, 7 or 8 days after the

application of the slug dose most of this nickel had been discharged in the effluent. This accumulation of nickel by the activated sludge during the first 24 hours following the addition of the slug dose would explain the decrease in the number of live bacteria released in the effluents of the slug dosed units, as was discussed previously, and the decrease in the oxygen uptake of these units; also the time that was required for the slug dose to be released in the effluent corresponded well with the time required for the viable bacteria in the effluent to return to a sizeable number after being significantly decreased by the addition of the slug dose. The fairly rapid release of the metal in the effluent (7-8 days) would account for the ability of activated sludge to recover from slug doses of nickel.

VI. CONCLUSIONS

From the results obtained in this study, several conclusions can be drawn:

1. The addition of nickel in the range of 1-10 mg/l affected the performance of activated sludge units, and resulted in higher effluent chemical oxygen demand (COD) and suspended solids.

2. The major portion of the increased COD values was caused by organic matter in the effluent suspended solids, rather than untreated soluble organics.

3. The units fed 2-10 mg/l of nickel supported substantially greater numbers of stalked protozoa than the unit which received no nickel (control), but very few free swimming ciliated protozoa or rotifers. More rotifers were found in the 1 mg/l nickel fed unit than in the control.

4. Considerably higher numbers of bacteria were found in the effluents from the nickel fed units than from the control, and more of these were viable.

5. All the activated sludge units fed 1-10 mg/l of nickel exhibited oxygen uptakes, both before and after the application of a 50 mg/l slug dose. In most cases, the uptakes of the nickel fed units were less than that of the control; this, coupled with the finding that live bacteria were present in the effluents, would indicate that the nickel fed units were functioning, but at a reduced rate as compared to that of the control.

6. About one-half of the nickel added to the units was lost in the effluent, and considerably more was released following the addition of the slug dose.

7. The almost complete absence of free swimming protozoa from the nickel fed activated sludge units appears to be responsible for the deterioration of the quality of the effluents. These organisms have the ability to move around, and would, therefore, be considerably more effective in ingesting suspended particles than the stalked forms.

8. Large amounts of nickel were released in the effluents following the addition of the slug dose, and this would account for the ability of activated sludge to recover from such slug doses.

VII. RECOMMENDATIONS FOR FUTURE RESEARCH

The results presented have established that nickel affected the microbial population of activated sludge; it adversely affected the growth of free swimming ciliated protozoa and rotifers, while it stimulated the growth of stalked protozoa. A 48 hour detention period was employed for the fill and draw activated sludge units which were operated on the total oxidation principle. Rotifer and protozoan counts should be made in the mixed liquor of a continuous flow nickel fed activated sludge unit with a 4-6 hour detention period. This would more correctly simulate the aeration time and operation principles used in most conventional activated sludge plants.

More bacteria, both dead and alive, were found in the nickel fed units than in the control. Simple stained slides were employed in determining the total number of bacteria in the effluent. Gram stained slides, or some other method of identification, should be used in additional work to determine if there is a change in bacterial predominance following the addition of nickel.

Continuous daily oxygen uptake studies of a short duration need to be made on nickel fed units concurrently with the identification of bacteria in an attempt to correlate a change in bacterial predominance with increases in oxygen uptake.

Runs of 40-46 days were employed in this study and indicated that the activated sludge continued to accumulate nickel without showing any signs of complete deterioration. Longer term studies may be used in order to determine to what level the nickel could be accumulated in the systems without causing complete destruction of the units.

Of interest would also be results of nickel determinations on the effluents and membrane filtered effluents to determine that proportion of nickel in the effluent which is incorporated in the increased effluent suspended solids, characteristic of nickel fed units.

The use of a synthetic medium to sustain nickel fed units in batch or continuous flow systems should be investigated. The addition of a synthetic carbon source, such as glucose, to an acclimated or an unacclimated system at time zero would provide data pertaining to the removal of glucose and COD, and would enable the determination of the oxygen uptake and mixed liquor suspended solids concentration. By using an appropriate control and by making microbial counts, the effects of nickel could be evaluated while the microorganisms were actively metabolizing.

The effects of a mixture of heavy metals on the microbial population of activated sludge should also be investigated.

BIBLIOGRAPHY

1. McDermott, G. N., Post, M. A., Jackson, B. N., and Ettinger, M. B., "Nickel in Relation to Activated Sludge and Anaerobic Digestion Processes," Journal Water Pollution Control Federation, 37, 163 (1965).

2. Barth, E. F., Ettinger, M. B., Salotto, B. V., and McDermott, G. N., "Summary Report on the Effects of Heavy Metals on the Biological Treatment Processes," Journal Water Pollution Control Federation, 37, 86 (1965).

3. Matthews, J. R., "The Effect of Nickel on Activated Sludge, " Thesis, University of Missouri at Rolla, 75 p. (With 10 figr., 14 tables), (1965).

4. McKinney, R. E., and Gram, A., "Protozoa and Activated Sludge," Sewage and Industrial Waste, 28, 1219 (1956).

5. Hill, H., "The Influence of Nickel on the Purification of Sewage by the Activated Sludge Process," Institute of Sewage Purification Journal and Proceedings, Part 1, 161 (1947).

6. Barth, E. F., Salotto, B. V., McDermott, G. N., English, J. N., and Ettinger, M. B., "Effects of a Mixture of Heavy Metals on Sewage Treatment Processes," Proc. 18th Annual Purdue University Industrial Waste Conference, Series 115, 616 (1964).

7. Stones, T., "Fate of Nickel During Treatment of Sewage," Institute of Sewage Purification Journal and Proceedings, Part 1, 252 (1959).

8. Moulton, E. Q. and Directo, L. S., "Standardized Procedure for Evaluation of Toxic Effects on Activated Sludge," Engineering Experiment Station, College of Engineering, The Ohio State University, Vol. XXXI, No. 3, (1962).

9. Dawson, P. S. S., and Jenkins, S. H., "The Oxygen Requirements of Activated Sludge Determined by Manometric Methods," Sewage and Industrial Wastes, 22, 490 (1950).

10. Heukelekian, H., and Gellman, I., "Studies of Biochemical Oxidation by Direct Methods," Sewage and Industrial Wastes, 27, 70 (1955).

11. "Standard Methods for the Examination of Water and Wastewater," 12th Ed., American Public Health Association, New York, N. Y. (1965).

12. Engelbrecht, R. S. and McKinney, R. E., "Membrane Filter Method Applied to Activated Sludge Suspended Solids Determination," Sewage and Industrial Wastes, 28, 1321 (1956).

13. Taylor, C. G., "Determination of Small Quantities of Nickel With Alpha-furildioxime," Analyst, 81, 369 (1956).

14. "Standard Methods for the Examination of Water and Wastewater," 11th Ed., American Public Health Association, New York, N. Y. (1960).

15. McKinney, R. E., "Microbiology for Sanitary Engineers," McGraw-Hill Book Co., Inc. New York, N. Y. (1962).

VITA

Virgil Eugene Carr was born on July 7, 1939 in Granby, Missouri to Mr. and Mrs. Harvey H. Carr. He received his primary and secondary education in Missouri and Illinois. He graduated from the Missouri Southern University in May, 1960 with an Associate of Science degree, and from the University of Missouri at Rolla in May, 1962 with a Bachelor of Science degree in Civil Engineering.

Following graduation, he was associated with Black and Veatch Consulting Engineers until he returned to the University of Missouri at Rolla for graduate study. He served as a Graduate Teaching Assistant in Civil Engineering during the 1965-66 academic year, and in June, 1966 was appointed a Graduate Research Assistant. On February 28, 1967 the author (with Dr. S. G. Grigoropoulos) presented portions of this thesis at the 1967 annual meeting of the Missouri Water Pollution Control Association in St. Louis.

He is a member of Sigma Xi, Chi Epsilon, Tau Beta Pi, Phi Kappa Phi, and Phi Theta Kappa honorary fraternities; and a member of the American Society of Civil Engineers, Missouri Society of Professional Engineers, and National Society of Professional Engineers. He is a registered professional engineer in the state of Missouri.

On January 20, 1964 the author and Miss Sandra H. Lindsay were married.